ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Fiscal Year 1973

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

ANNUAL REPORT

July 1, 1972 through June 30, 1973

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OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

In the preceding year the research program of the Institute was strengthened in the mutagenesis and developmental biology areas; but projected reductions in funds and personnel for Fiscal Year 1974 have made it necessary to review plans for the development of the research program for the Institute. Rather than support meagerly all components of a well-rounded program in environmental health sciences together with necessary support and ancillary services, resources of NIEHS will be concentrated on the development of strong research programs in reproduction and development, mutagenesis and pharmacology-toxicology, together with support and collaboration of the disciplines of synthetic and analytical chemistry, pathology, histology and electron microscopy, and veterinary medicine. Research on physical factors in the environment as well as biometry and experimental statistics are being continued at their current modest levels. A detailed analysis of these considerations is presented in the Intramural Research Report.

CONSULTATION AND LIAISON

The liaison activities of NIEHS have been continued to relate to scientific programs and are effectively carried out on both a scientist-to-scientist basis and a more official basis. The Director and his staff have served as advisors to the Office of the Secretary, HEW, and represent the Department in the consideration of current environmental problems including smoking and health, lead, organic pollutants, mercury and asbestos.

The staff of the Institute has cooperated with the National Institute of Occupational Safety and Health by reviewing critically the drafts of several criteria and standards documents to be adopted by HEW and the Department of Labor. The Director formally represents this Institute as observer on the Advisory Board for the National Center for Toxicological Research, which insures exchange of information in the closely related missions of these two organizations.

The OST-CEQ Ad Hoc Advisory Committee on Environmental Research, the Chairman of which was the Director of NIEHS, surveyed the various environmental research activities and information sources within the Federal Government and has submitted its report. Currently, alternatives are being considered to determine how this activity can be continued to meet the need for mechanisms whereby coordination may be developed in making regulatory decisions in public health.

In September, a parallel Committee on Ecology submitted its report for CEQ on Federal Research in Ecology. The Associate Director for Scientific Information and Communication continues to participate in the activities of this committee.

SCIENTIFIC CONFERENCES AND WORKSHOPS

The favorable responses to previous working conferences on current environmental problems sponsored by NIEHS have encouraged the Director to extend the series of conferences and workshops as a means of assessing current state of knowledge and possible impact on human health and identifying research that should be undertaken or sponsored by participating agencies and organizations. Conferences sponsored during Fiscal Year 1972 are:

September 6-7, 1972	Phthalate Esters Conference -- The Carolina Hotel, Pinehurst, North Carolina [approximately 100 scientists from the United States, Canada, and England attended]
February 1, 1973	"The Effects on Human Health of Asbestos" -- Combined Clinical Staff Conference, NIH, Bethesda, Maryland
March 8-9, 1973	"Heavy Metals in the Environment", Symposium -- Governors Inn, Research Triangle Park, North Carolina
April 2-3, 1973	"Chlorinated Dibenzodioxins and Dibenzofurans" -- Governors Inn, Research Triangle Park, North Carolina [approximately 100 scientists from the United States, Canada, and England attended]
April 25-28, 1973	"Evaluation of Chemical Mutagenicity Data in Relation to Population Risk", Workshop -- Governors Inn, Research Triangle Park, North Carolina [approximately 75 scientists from England, Sweden, Canada, Germany, and the United States participated]

These conferences provide a face-to-face interchange between scientists working in the same broad problem areas. In addition, through its experimental journal, Environmental Health Perspectives, the Institute publishes the proceedings of these conferences so as to insure maximum communication of research findings. Reactions from those who read Perspectives indicate our efforts in this direction are successful.

INTERNATIONAL PROGRAMS

The Director, his immediate staff, and a number of the Institute's scientists are actively participating in the program of the Joint Committee on Health Cooperation of the United States-Soviet Health Exchange in accordance with the agreement between DHEW, USA, and the USSR Ministry of Health signed in Moscow on March 21, 1972.

In August 1972, during the visit of the USSR Ministry of Health Delegation to the U.S., Dr. Sidorenko and Dr. Rall further discussed plans for the implementation of this exchange program.

In September 1972, at the invitation of the U.S.S.R., a U.S. environmental health delegation attended sessions of the XVI All Union Congress of Hygienists and Public Health Physicians held in Moscow and visited Soviet Institutes working in areas of mutual interest. Dr. Rall, head of the U.S. delegation, was accompanied by two NIEHS scientists, Dr. Robert Drew and Dr. Donald McRee, and Dr. Norton Nelson of New York University.

On October 26, 1972, Dr. Aleksandr V. Pavlov, Ministry of Health, U.S.S.R., visited the NIEHS and spent the morning touring laboratories and the afternoon discussing the environmental health exchange program, particularly the itinerary for the forthcoming visit of the U.S.S.R. environmental health delegation.

At a meeting held at NIEHS from January 22 to January 29, 1973, the U.S.S.R. delegation of five scientists, headed by Professor G. I. Sidorenko, and a group of U.S. scientists, headed by the NIEHS Director, exchanged views on the proposed cooperative program and reached an agreement to conduct joint investigations on the following problems:

- Methodological basis for the assessment of the biological effect of inhaled chemicals;
- Methodological basis for the assessment of the biological effect of orally introduced chemicals; and
- Scientific basis for the assessment of the complex biological effect of chemicals (inhaled and introduced orally).

Arrangements are being made for exchange of specialists to work on specific topics agreed upon during the visit.

During the week of March 26, 1973, the Director participated in the US-USSR Health Exchange Joint Committee meeting in Bethesda, Maryland.

Under the US-USSR Environmental Protection Agreement, the Director of NIEHS is Co-Chairman of the segment on the "Genetic and Biological Effects of Pollutants". A delegation of Soviet scientists, led by Academician Dubinin, is expected to visit NIEHS June 3-17. Primary areas of cooperation will be in mutagenesis testing, heavy metal toxicology, and (with the National Environmental Research Center of the Environmental Protection Agency, Research Triangle Park) community health effects of air pollution.

Under a bilateral agreement with France, reciprocal visits are arranged for study of basic pulmonary reactions to inhaled pollutants. Dr. John Knelson, Environmental Protection Agency, is visiting France, and Dr. Q. T. Pham, Unit de Recherche de Physio-Pathologie Respiratoire, Nancy, will be visiting the United States for about six weeks in each case.

As a part of the US-Japan Cooperative Medical Sciences Program, Dr. Frederick J. de Serres is serving as U.S. Chairman for the Panel on Methods to Evaluate Environmental Mutagenesis and Carcinogenesis. Following a meeting of the Joint Committee in Tokyo, a symposium on mutagenic and carcinogenic effects of environmental pollutants was held August 25-27, 1972.

OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM

OFFICE OF ASSOCIATE DIRECTOR FOR PROGRAM
Summary Statement

During fiscal year 1973 the Office of the Associate Director for Program initiated a technological surveillance activity with the help of two chemical engineers who became available through the National Science Foundation as part of the program of the President's Internship in Science and Engineering. With their help, a survey was made of potential health problems that may exist now or become real in the near future because of increased manufacture of certain products or modification of processes of production. It was also thought of importance to evaluate the effects of actions taken by Governmental agencies in curtailing or restricting the use of some hazardous substances which will lead to their replacement by other chemicals presumed to be less toxic but which may as yet not be adequately tested.

The first reports that were developed dealt with the types of chemicals used in the manufacture of plastics and artificial rubber and with fuel additives, particularly those suggested for replacement of tetraethyl lead in gasoline.

Because of the need for new antibacterial compounds to replace hexachlorophene or mercury containing products, a search for toxic properties of the proposed replacements brought out evidence that many of the available compounds show some adverse effects.

These surveillance programs will not be closed, but will remain open for additions and corrections whenever available.

To supplement our industrial and environmental surveillance activity, a contract has been supported which searches the literature, obtains reports and makes contact with experts in and outside industry for information on the chemical and physical properties of metallic compounds reaching man's environment in air, water, food or consumer products which may prove hazardous to man. The office is also responsible for a contract on the interaction of environmentally important toxic metals in tissue culture systems.

This office is responsible for supervising contract research on the acute toxicity of certain detergent builders, polyphosphate with and without nitrilotriacetate or sodium carbonate and metasilicate. It also supervises a contract activity exploring the effects of inhalation of uranium ore dust and radon daughters in combination with cigarette smoke or diesel engine exhaust. The studies utilizing hamsters have been completed and are in the process of final analysis, while those involving beagle dogs are still in progress and will require additional time.

This office has continued to give advice to HEW and other Governmental agencies on hazardous substances in specific situations. The Associate Director gave advice and criticism to EPA on their criteria and standards documentation regarding health hazards of lead in gasoline on request by HEW and was invited to be a member of the Subcommittee on Research and Statistical Issues of the Interdepartmental Coordinating Committee on Childhood Lead Paint Poisoning.

As a member of the National Academy of Sciences Panel on Vapor Phase Organic Air Pollutants, he participated in the preparation of a document and in writing a chapter on Synergistic and Inhibitory Effects. This document is prepared for use by the EPA.

On request by HEW, he transmitted advice and criticism through channels on a document: National Emissions Standards for Hazardous Air Pollutants: Asbestos, Beryllium and Mercury.

On request by the National Institute of Occupational Safety and Health, the Associate Director served on a Review Committee on Criteria Documentation for an Occupational Exposure Standard for Fibrous Glass and reviewed other documents for criteria standards on arsenic and sulfur dioxide.

On invitation by the Editor of the Swedish journal AMBIO, he wrote an article entitled Interaction of Fluorescent Whitening Agents and Ultraviolet Radiation, and attended a conference sponsored by EPA on the hazards of fluorescent whiteners where Government and industry scientists discussed freely their research results.

The Associate Director also attended other EPA or NIEHS sponsored conferences, as the ones on plasticizers and polychlorinated dibenzodioxins, and helped in the planning of a 1974 symposium on Health Consequences of Environmental Controls.

This office collaborated with the USDA on the potential health hazards associated with consumption of late blight infected potatoes and interested competent scientists in taking on this problem and clarifying the facts. It also will cooperate in an Interagency Task Force on Food Safety Monitoring and has expressed interest in studying health problems related to reutilization of animal waste. The Associate Director gave on invitation a lecture on Toxic Fungal Metabolites in Food and Feed at Temple University.

This office continued to assist the Clearing House for Smoking and Health (HEW) on critically reviewing the chapter on cancer in the publication on Health Consequences of Smoking and the Associate Director attended the meeting of the Subcommittee of the Joint Committee on Tobacco and Health, held in St. Thomas, Virgin Islands, and attended the Annual Tobacco and Health Workshop Conference in Lexington, Kentucky. He also serves as consultant to the Advisory Committee on Smoking and Health to the Kentucky Tobacco Research Board.

By invitation from the National Research Council, the Associate Director participated in the Conference on Principles of Protocols for Evaluating

Chemicals in the Environment for which he was Panel Chairman on Carcinogenesis. On invitation, he also lectured on Environmental Chemicals as Carcinogens, Teratogens and Mutagens in a course given at Colorado State University at Fort Collins, Colorado, and similarly lectured on Chemical Carcinogenesis on several occasions at Duke University, Durham, North Carolina. The invited chapter on Chemical Carcinogens, Mutagens, and Teratogens in the book Environmental Problems in Medicine, Dr. William D. McKee, Editor, should be in print shortly.

The Associate Director was active as Coeditor of the experimental journal Environmental Health Perspectives of which four issues were prepared during this fiscal year. He remained on the editorial board of Environmental Letters, Chemico-biological Interactions, and reviewed articles for Science and other journals on request.

He continued his activity as consultant to the International Agency for Research on Cancer and contributed advice to the chapters on metals to be published in the second monograph: 'Evaluation of Carcinogenic Risks. He acted as Chairman of the Working Committee Evaluating the Risk for Man of Exposure to Polycyclic Aromatic Hydrocarbons and wrote the preamble: Human Cancer and Exposure to Polycyclic Aromatic Hydrocarbons for this second monograph. The first monograph, the result of last year's effort, has appeared in print and serves as baseline for many criteria and standards documents. The Associate Director will spend several months consulting with IARC on continued activity of the Committee Evaluating Carcinogenic Risks to Man.

In a separate activity the Associate Director was asked by the Director of the Division of Environmental Health, WHO, to consult and collaborate in the production of a document: Environmental Health Criteria and Standards, which, if accepted by the collaborating agencies of several countries, would be the basis of a collaborative effort to obtain excellent information on criteria and serve each country in promulgating their own standards. Following the acceptance of the document, the preparation of Outlines for Criteria Development was undertaken by WHO and contributions were made by the Associate Director in several areas.

Because of the importance of this activity not only for WHO and the collaborating agencies in other countries, but specifically for the NIEHS in its effort to communicate its findings and obtain information from other laboratories on health problems due to environmental contamination and pollution, the Associate Director is prepared to spend 14 months at Geneva to collaborate on the implementation of the effort and to streamline the contributions of information by the participating agencies in a dozen countries.

Serial No.: NIEHS-ADP-001
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "An Investigation of Interaction of Environmental Pollutants in Causing Porphyria"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Hans L. Falk, Ph. D.

OTHER INVESTIGATORS: Sandra Jordan

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	0.1
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Hexachlorobenzene, other chlorinated hydrocarbons (PCB's, polychloronaphthalenes, polychlorodibenzodioxins), allyl alkyl acetamide and related chemicals or drugs, as well as lead salts, will be studied for their production of porphyria in rats.

METHODS EMPLOYED: The appearance and disappearance of porphyria will be determined spectroscopically by determination of uro- and coproporphyrin in the urine at timed intervals following administration of the chemicals by oral intubation.

MAJOR FINDINGS: Hexachlorobenzene (0.2% in the diet) produced porphyria in rats after two to five weeks. After removal of hexachlorobenzene from the diet the disappearance of porphyria may take a varied length of time from 8 to over 20 weeks. The output of uro- and coproporphyrin runs parallel at times, but may be reciprocal as the effect wears off, which needs further study.

Feeding lead acetate in drinking water at the 0.1% level did not produce prophyria or interfere with porphyria production in hexachlorobenzene fed rats. At higher concentrations of lead the animals refused the water. Intubation of lead acetate by gavage at a dose of 40 mg each on two successive days produced a significant drop in urinary porphyrins in hexachlorobenzene-pretreated animals. In non-pretreated animals the administration of lead

acetate produced a rapid rise in coproporphyrins in the urine which disappeared rapidly in 10 days after single administration.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hexachlorobenzene is an environmental contaminant which has proved to be porphyrogenic for experimental animals as well as man. This chemical can be formed from a number of chemicals possessing a carbon chlorine bond by microorganisms and it also appears in man's environment as a result of industrial activity. It is encountered in the presence of other related chemicals like the PCB's, the chlorinated hydrocarbon pesticides and such compounds as the polychlorodibenzodioxins and furans. Not all of them are porphyrogenic. Other unrelated chemicals as well as a number of environmental breakdown products and pharmaceuticals can produce porphyria by the same or different mechanisms. It is, therefore, of importance to examine the various potential environmental porphyrogens and evaluate their mode of action and interaction.

PROPOSED COURSE: Chemicals that are suspect of being porphyrogenic will be given to rats alone or in combination with other porphyrogens to determine the significance of this toxic action for man. They will be sorted by chemical structure and by biochemical action to obtain a better classification of porphyrogenic chemicals.

Serial No.: NIEHS-ADP-002
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Technology Surveillance, Use Patterns, and Toxicity of the Chemical Process Industries"

PRINCIPAL INVESTIGATOR: Warren T. Piver, Ph. D.

OTHER INVESTIGATORS: Hans L. Falk, Ph. D. and Robert E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To provide background information about toxic chemical market products of the chemical process industries. This information is to include chemistry of the compound, reason for its use, use patterns, volume of production, transport mechanisms in the environment, toxicity of the compound, estimation of chronic exposure levels to the compound, and projected short-term and long-term forecasts of production volume of the compound.

METHODS EMPLOYED: Literature review, classification of CPI by major product lines, i.e. plastic industry, rubber industry, petrochemical, etc. modeling of environmental processes, technology forecasting, industrial inquiry, and interviews with biological scientists.

MAJOR FINDINGS: A. Organotin stabilizers in PVC plastics: Stabilizers are essential additives for halogenated plastics such as PVC to prevent dehydrochlorination degradation of the product in its use pattern. The dibutyl and dioctyl tin stabilizers are among the most effective compounds for stabilization of PVC. In flexible PVC medical devices such as blood bags, connecting tubing, and cathethers, organotin stabilizers can be extracted from the device as a soluble component of the extracted plasticizer. Tissue culture tests of organotin compounds have shown them to be much more toxic than the plasticizers in which they were dissolved. Flexible PVC is also used as a meat wrap and for making food containers. Disposal of both flexible and rigid PVC items is usually by incineration so that the compound may end up in the air or in the waste water from stack scrubbers. Market for organotin stabilizers should continue to rise since PVC production shows no signs of diminishing.

B. Alternatives to leaded gasoline: Two major proposed alternatives are a higher percentage of aromatics in gasoline or the use of substitutes for tetraethyl lead (TEL). In uncontrolled exhaust systems which do not use either the catalytic or thermal converter, emission control devices, or are not properly maintained, a higher percentage of polycyclic aromatics will be emitted into the air from a higher percentage of aromatics in the gasoline. The most effective alternative for TEL is methylcyclopentadienyl manganese tricarbonyl. This additive itself has a polytropic toxicity with 1 ppm in air affecting the central nervous system and giving rise to early histological changes in the respiratory tract. The combustion products of this additive would probably be either MnO, MnO_2, Mn_2O_3 or Mn_2O_4. In the air, these oxides can react to form $MnSO_4$, $Mn_2S_2O_6$, and $Mn(NO_3)2$. In fog, with about 1.7 ppm SO_2, and 0.0002 ppm $MnSO_4$, $MnSO_4$ rapidly catalyzes the formation of H_2SO_4 from SO_2. Chronic exposure of manganese workers to MnO_2 dust at concentrations of 1 - 5 mg/m^3 has produced chronic manganese poisoning, a disease of the central nervous system, and manganese pneumonitis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These surveillance programs will provide necessary information about the products and use patterns of chemicals of the CPI and can be used in planning research programs for the Institute.

PROPOSED COURSE: This work will continue to examine other chemical process industries to determine what and how much toxic or potentially toxic chemicals are being transported into the environment. Emphasis will continue to be given to non-chemically held additives for plastics and rubber products because these two particular industries are expanding at such a rapid rate both in volume and number of product uses. Attention will also be given to determining better environmental transport models and modeling techniques and better methods of technology forecasting. Because of industrial and cultural habits, a critical analysis of present and future waste disposal and recycling processes which are intended to cure pollution problems will be carried out.

PUBLICATIONS

Piver, W. T.: Organotin compounds, industrial applications and biological investigations. Environm. Hlth. Persp. No. 4, June 1973.

Serial No.: NIEHS-ADP-003
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazards From Flame Retardant Compounds"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.10
Professional:	.10
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To obtain from the literature and other sources information relating to possible health hazards resulting from the use of flame retardants. Hazards from the retardant itself and from its decomposition products should be considered.

MAJOR FINDINGS: Several of the additive types of flame retardants are: antimony compounds, inorganic salts, halogen compounds, phosphorous compounds, and nitrogen compounds. In addition, there are some thermoplastics which are produced to be inherently flame retardant, such as compounds which use organo-metallics directly in their chemical formulation. The potential environmental health hazards could arise either from the flame retardants themselves or from their breakdown products.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of this survey may point out an area of potential environmental health hazard.

PROPOSED COURSE: This survey is currently continuing. Among the areas of interest are: the types of fire retardants considered for future use, the type currently used, the types of reactions involved during combustion, the emission of toxic breakdown products during this process, the stability of the compounds during normal use, and the related environmental health hazards.

Serial No.: NIEHS-ADP-004
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazard of Hexachlorophene Substitutes - Antimicrobials in Cosmetics"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.15
Professional:	.15
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To obtain, from the literature and other sources, information relating to the possible health hazards resulting from the antimicrobials substituted for hexachlorophene in cosmetic uses.

MAJOR FINDINGS: The information relating to the hazards of use of the antimicrobials substituted for hexachlorophene seem either not to be complete, even for the older established compounds, or not to have been fully disseminated. Among the compounds considered were halogenated salicylanilides and halogenated carbanilides. Also mentioned was a newer compound, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, which showed potential as a broad spectrum antimicrobial agent. This compound was noted to contain as impurities 2,8-dichlorodibenzodioxin and 2,4,8-trichlorodibenzodioxin.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of the survey point out an area of potential environmental health hazards to the public.

PROPOSED COURSE: A short report has been written, but the topic will remain open for continued surveillance.

Serial No.: NIEHS-ADP-005
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Continuing Surveillance for New Potential Environmental Health Hazards"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.15
Professional:	.15
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To continually survey the literature for new products, product modifications, and manufacturing process alterations that may present a potential of becoming or generating a health hazard.

MAJOR FINDINGS: Among areas that have been singled out as a result of this continuous surveillance for further investigation are: antimicrobials in cosmetics, blowing agents, flame retardants, fibrous reinforcements in plastics, fillers for plastic compounding, aerosols, and paints and coatings.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The result of this constant surveillance is that new areas of potential environmental health hazards are discovered.

PROPOSED COURSE: The surveillance of technical reviews and various technical journals will continue with respect to new potential environmental health hazards.

Serial No.: NIEHS-ADP-006
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazards From Paints"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.13
Professional:	.13
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To obtain from the literature and other sources information relating to the possible health hazards arising from the various components used in the formulation of paints.

MAJOR FINDINGS: Paint and coating products contain many components, other than the relatively well known heavy metal pigments, that may be toxic.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of the survey may point out an area of potential environmental health hazards.

PROPOSED COURSE: This topic is currently under investigation. Of interest in this survey are the compounds that go into paints, such as: solvents, resins, drying agents, emulsifiers, pigments, extenders, antimicrobials, etc. These components are being considered with respect to their potential health hazards.

Serial No.: NIEHS-ADP-007
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazard From Chemical Blowing Agents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.15
Professional:	.15
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To obtain, from the literature and other sources, information relating to the possible health hazards resulting from the use of chemical blowing agents in plastic and rubber foams and from the handling of these finished products. Both acute and chronic toxicity were to be considered.

MAJOR FINDINGS: The nine major blowing agents investigated were: azobisformamide, dinitrosopentamethylenetetramine, N,N'-dinitrosoterephthalamide, p,p'-oxybis (benzenesulfonylhydrazide), p-toluenesulfonylsemicarbazide, p-toluenesulfonylhydrazide, azobis(isobutyronitrile), diazoaminobenzene, and benzenesulfonylhydrazide. Of these compounds, diazoaminobenzene is the only one that has been proven to be carcinogenic in animal studies. The potential health hazard would most likely arise not from the blowing agents themselves, but from the decomposition residues remaining in the foam.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of the survey may point up an area of potential environmental health hazard.

PROPOSED COURSE: A report has been completed, but the topic will remain open for continued surveillance of these types of compounds.

Serial No.: NIEHS-ADP-008
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazard From Fillers for Plastic Compounding"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.10
Professional:	.10
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To obtain from the literature and other sources information related to the materials potentially hazardous to health, used or considered for use, as fillers for plastic compounding.

MAJOR FINDINGS: There is a wide range of compounds used as fillers. Among these compounds are silicas, calcium carbonate, metallic oxides, various other inorganic compounds, as well as metal powders, carbon black, cellulosics and carbon. Among currently used fillers that could be hazardous would be barium ferrite, beryllium oxide, or lead powder.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This survey points out another source of materials that are a potential environmental health hazard to the public.

PROPOSED COURSE: A preliminary report has been written, but the topic will remain open for continued surveillance.

Serial No.: NIEHS-ADP-009
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazards From Aerosols"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.12
Professional:	.12
Other:	.00

PROJECT DESCRIPTION

OBJECTIVE: To obtain from the literature and other sources information relating to the possible health hazards resulting from the widespread consumer use of aerosols.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This survey may point out an area of potential environmental health hazards.

PROPOSED COURSE: The areas of investigation are the composition and use of aerosol products; the toxicity, both acute and chronic, of the compounds; the potential exposure of the public; and, hence, the potential health hazards engendered by the products.

Serial No.: NIEHS-ADP-010
1. Associate Director for Program
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Potential Health Hazards From Fibrous Reinforcements in Plastics"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. E. Munson, Ph. D.

COOPERATING UNITS: None

MAN YEARS

Total:	.1
Professional:	.1
Other:	.0

PROJECT DESCRIPTION

OBJECTIVE: To obtain from the literature and other sources information related to the materials potentially hazardous to health, used or considered for use, as fibrous reinforcements in plastics.

MAJOR FINDINGS: Among the fiber reinforcing materials used are: asbestos, boron, carbon, ceramic, glass, graphite, jute, sisal and whiskers. Several synthetic fibers such as nylons, polyethylene terephthalate and polyacrylonitrile are used. Asbestos and glass fiber reinforced products with 5 to 60% and 35 to 40% fibers, respectively, are in wide use.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This survey points out another source of materials that are a potential environmental health hazard to the public.

PROPOSED COURSE: A preliminary report has been written, but the topic will remain open for continued surveillance.

CONTRACTS

AMERICAN ACADEMY OF PEDIATRICS, Evanston, Illinois (NIH-NIEHS-72-2903)

TITLE: "Research on Early Detection of Environmental Agents that Induce Diseases in Children"

CONTRACTOR'S PROJECT DIRECTOR: S. L. Harrison, M.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D.

DATE CONTRACT INITIATED: June 1, 1972

CURRENT ANNUAL LEVEL: $33, 429

PROJECT DESCRIPTION

OBJECTIVES: The contractor will establish and manage programs at three medical centers focusing on the early detection of environmental agents that induce congenital malformations or other diseases in children.

METHODS EMPLOYED: Records are prepared at the three medical centers by selected medical students relative to pertinent environmental exposure histories of the child and the parents. These exposure histories, as well as pertinent clinical information, are summarized by the medical center Principal Investigator and submitted to the AAP Committee on Environmental Hazards. The Committee reviews the summaries, prepares relevant commentary, and forwards the information to NIEHS as quarterly progress reports.

MAJOR FINDINGS: Due to the considerable delay experienced in completing transfer of contract funds from the contractor (AAP) to the three clinical pediatric centers, it is too early to report any findings for this study.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Detection and identification of environmental stressors continues as an important biomedical research endeavor. New leads for laboratory and epidemiologic investigations are being generated through this system which provides a mechanism for monitoring the fetus and newborn for evidence of deleterious effects due to various environmental influences.

PROPOSED COURSE: This contract will be extended for 16 months to provide for continued monitoring at the three participating pediatric centers.

BATTELLE NORTHWEST-PACIFIC NORTHWEST LABORATORY, RICHLAND, WASHINGTON
(Interagency Agreement Between NIEHS and AEC, #300A00444)

TITLE: "Study of the Combined Effects of Smoking and Inhalation of Uranium Ore Dust, Radon Daughters and Diesel Oil Exhaust Fumes in Hamsters and Dogs"

CONTRACTOR'S PROJECT DIRECTORS: J. F. Park, Ph.D. and B. O. Stuart, Ph. D.

PROJECT OFFICER (NIEHS): Hans L. Falk, Ph. D.

ASSOCIATE PROJECT OFFICER: Phillip J. Walsh, Ph. D.

DATE CONTRACT INITIATED: June 1, 1968

CURRENT ANNUAL LEVEL: $336,000

PROJECT DESCRIPTION

OBJECTIVES: As a major part of an integrated research program on the effects of exposure to radon daughters on the development of lung cancer in uranium miners, the following animal studies were initiated:

1. Groups of hamsters were exposed to the following combinations:

    a. radon daughters on room air nuclei,
    b. radon daughters and carnotite ore dust,
    c. diesel engine exhaust alone,
    d. diesel exhaust fumes, radon daughters and ore dust,
    e. carnotite ore dust.

Concentrations of radon daughters, composition of ore dust and concentrations of other toxic agents in the diesel oil exhaust fumes were carefully controlled. After being exposed to predetermined cumulative dosage levels, the animals were maintained for life for the observation of pathological changes, particularly lung cancer and emphysema.

2. To test specifically the possible synergistic effects of radon daughters and cigarette smoking, studies were initiated on three groups of 18 dogs as described below:

    a. dogs exposed to radon daughters and carnotite ore dust exposure for four hours per day, 7 days per week at concentrations of 15 nanocuries per liter; with sham smoking;

b. dogs exposed to radon daughters and ore as in (a), plus two working shifts of smoking (total of 10 cigarettes per day with resting on alternate hours).

Regular clinical, radiological and radiographic examinations will be made during and after the smoking and radon exposures. The dogs, except for one in each group which will be sacrificed for early histopathological studies, will be held for their life span with observations for pulmonary changes and lung cancer as the end point.

MAJOR FINDINGS: Phase II of the hamster studies has been terminated. It consisted of six groups of male hamsters that simultaneously had daily 6-hour inhalation exposure to laboratory air (controls), 1200 WL of radon daughters, 1200 WL radon daughters with 15 mg/m^3 uranium ore dust alone, diesel engine exhaust and diesel engine exhaust with 1200 WL radon daughters and uranium ore dust. Hamsters showed respiratory tract lesions covering a spectrum ranging from normal lungs in control hamsters to metaplasia of bronchial and bronchiolar epithelium and adenomatous hy perplasia of bronchioloaveolar epithelium (cuboidal metaplasia or bronchiolization) in hamsters in the experimental groups. The most frequent finding was that of focal accumulations of particulate-laden macrophages adjacent to small bronchioles and vessels; where such particle deposits were heaviest, they were frequently accompanied by septal cell hyperplasia. This macrophage accumulation was found in almost all of the hamsters exposed to diesel engine exhaust alone or with radon daughters and uranium ore dust. Similar accumulation of macrophages was found in about 50% of the hamsters that inhaled uranium ore alone or with 1200 WL radon daughters. Hyperplasia and metaplasia appeared after 5-6 months of exposures, increasing sharply thereafter; adenomatous lesions also appeared after six months. Mortality and body weight of experimental groups showed no significant difference from controls.

The proliferative lesions observed earlier in bronchiolar and alveolar areas progressed to carcinoma in situ and in some animals exposed to 1200 WL of radon daughters and uranium ore dust became invasive cancers. The animals exposed to diesel exhaust or uranium ore dust alone did not show any cancers, but had other severe pulmonary pathologic changes including emphysema, fibrosis, inflammatory reactions and epithelial squamous metaplasia. The complete analysis of the pathologic findings can now be undertaken as all animals have died.

Sixty beagle dogs continue to receive daily inhalation exposures to cigarette smoke and/or 600 WL of radon daughters with uranium ore dust. These animals have received 42 months of exposures to date. Determinations of carboxyhemoglobin levels in all dogs are being correlated with individual smoking patterns. No significant differences from controls have appeared in respiratory rates and volumes or body weights; total white blood cell counts and absolute segmented neutrophil levels have risen higher in the group receiving cigarette smoke plus radon daughters with ore.

Pulmonary lavage showed greater inflammation in those dogs on cigarette smoke and exposure to radon daughters and uranium ore dust than in those on cigarette

smoke alone. This greater inflammatory response was also seen in dogs on alpha-radiation and ore dust without cigarette smoke inhalation. In one dog that died after 33 months of exposure, fibrotic lesions were detected in the lungs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These experiments were designed to determine, in both a short-lived animal, the hamster (life-span 2-3 years), and a long-lived species, the dog (life-span 12-13 years), the possible synergistic effects of chemical agents--dust, cigarette smoking and diesel exhaust fumes--operating singly and in concert with exposure to radon daughters upon the production of lung cancer and other lung diseases in animals. To the extent possible in the laboratory, the exposure conditions are designed to simulate those to which uranium miners have been exposed, and in whom an increased incidence of lung cancer has been observed.

PROPOSED COURSE: The hamster experiments have come to an end and results are being analyzed. The dogs will continue to be exposed to cigarette smoke, uranium ore dust and radon daughters singly or in combination.

BATTELLE PACIFIC NORTHWEST LABORATORIES, RICHLAND, WASHINGTON (NIH-72-2043)

TITLE: "Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NI HS): E. O. Oswald, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $100,490

PROJECT DESCRIPTION

OBJECTIVES: To determine, using advanced radiotracer, spectroscopic and chromatographic techniques: (1) the rate and extent of uptake of metals from soils by representative grass and broad leafed plants, (2) the translocation of metals in plants, (3) the effect of metals on the soil microbiota and soil microbial process, (4) the nature of metal bond types and chemical forms of metal metabolites in plant and microbial tissues, and (5) the potential for the soil formation of organometal complexes, subsequent uptake of these compounds by plants and entrance into the human food chain; inherent in these objectives is the need to develop suitable methods for determining the chemical forms of metals, and their fate in soils, plants and microbial tissues.

METHODS EMPLOYED: Chromatography (TLC, column, GLC and high pressure liquid), spectroscopy (U.V., Visible, IR, NMR and Mass) and radiotracer techniques.

MAJOR FINDINGS: The metals employed in these investigations include silver, arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, tungsten and zinc.

Of the metals studied, copper, iron, manganese, molybdenum and zinc are required in small quantities for normal plant growth. Within this group of essential metals only copper and molybdenum resulted in large plant growth reductions upon amendment to soil. More importantly, the levels of molybdenum taken up in the plants were sufficiently high to cause toxicity in grazing animals. Autoradiography indicates that iron, manganese and zinc were generally distributed in shoots of the soybeans.

A second group of elements, including cadmium, cobalt, nickel and tungsten, was taken up in significant quantities but did not result in marked growth reduction. These elements were shown to be distributed throughout the soybean plants with concentration in different parts of the plant, depending upon the element. None of these metals showed any exclusion from the top by accumulation in the lower stem. The main danger of this group of metals may lie in the fact that plants accumulate large quantities of these metals but the plants are not poisoned by these materials and thus grow to produce contaminated crops.

The remaining metals studied were toxic to plants. Arsenic and thallium both showed an increase in tissue levels which nearly paralleled both the increase

in soil concentration levels and the reduction in yield. Arsenic was distributed throughout soybean shoots whereas thallium was excluded from the upper stem and leaves by an operational mechanism in the lower vascular system. The toxicity of silver, mercury, lead and thallium may be reduced because these metals are excluded from most of the plant tops. In contrast, arsenic and tin are not excluded by accumulation in the lower stem and thus reached areas of photosynthesis.

In relationship to the effect of those metals on soil components, only lead and tungsten did not have an appreciable effect on soil respiration and microbial population. Cobalt affected only microbial population only at 100 µg/g of soil. Manganese did not affect soil respiration but resulted in changes of soil microorganism at all concentrations of the metal. Arsenic was the only metal tested that effected changes in soil respiration at all tested concentrations of the metal without changing the population of total aerobic bacteria. The metals—tin, mercury, silver and iron—appear to affect soil respiration and soil bacteria at all levels employed.

Preliminary characterization of ammonium acetate extracts of the soybean shoots which have been grown under amended concentrations of the various metals indicates that the relative solubility of metals in the initial extract ranges from less than 10% for silver to 90% for thallium. Cobalt, nickel, tin, thallium, tungsten, and zinc exhibited the greatest solubility while chromium, iron, mercury, lead and silver exhibited the least solubility. Partitioning of the aqueous extract into molecular weight fractions indicates that for the molecular weight fraction greater than 10,000, the metal distribution varies from 3% for the original extracted thallium to a high of 95% for iron with a mean recovery of 51%. For materials with a molecular weight of 500-10,000, the recovery of metal in this range varies from 5% for iron to 49% for zinc with a mean concentration of 29%. For the fraction containing materials of molecular weight less than 500, this extract contains metals in concentrations ranging from 2-3% for molybdenum and tungsten to 90% for thallium with a mean of 27%.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides, desiccants or as contaminants of fertilizers. The importance of soil and plants in this pathway has been underscored by the spectre of metal conversion to more soluble and more toxic heavy metal complexes which may subsequently be taken up by plants and thereby enter directly into human foodstuffs. Particular attention should be given to determining the mechanisms for converting inorganic metals to organic complexes and the extent of this conversion. This information should provide a sound basis for estimating the potential hazard arising from the continued use of these materials and may provide the key to remedial measures.

PROPOSED COURSE: Research emphasis will be directed toward completing the plant and microbial studies underway and determining the forms of metals in plant and microbial tissues. Principal emphasis will be placed on separating inorganic and organic forms and on identifying low molecular weight organometal compounds in plant tissues. Mixed cultures of microorganisms exhibiting resistance to metals in soil as determined from microbial count and soil

respiration rates will be isolated using enrichment techniques and culture *in vitro*. The distribution of the metal in exocellular solution and within the cell will be measured with the form of the metal being determined by methods similar to those employed in plant studies.

BROOKHAVEN NATIONAL LABORATORY
Upton, New York

TITLE: "Environmental Mutagen Proposal Utilizing Higher Plants as Test Systems"

CONTRACTOR"S PROJECT DIRECTOR: A. H. Sparrow, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED: June 6, 1972

CURRENT ANNUAL LEVEL: $105,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of the proposed research are: (a) to demonstrate the usefulness of and further develop the Tradescantia somatic mutation test system as a quantitative monitor in environments containing known or suspected mutagens; (b) to use special genetic clones of Tradescantia to establish the relative mutagenicity of a limited number of mutagens (primarily in gaseous form), as to magnitude of effect, specificity of action, and possibility of reversion; (c) to look for evidence of genetic repair of DNA injury produced by chemical mutagens and to look for possible threshold levels below which induced mutation rate is negligible; and (d) to determine in greater detail the time and dose response for several loci for one selected gaseious mutagen, comparing these with presently available radiation data and with results observed in animal cells.

METHODS EMPLOYED: Two different clones of Tradescantia (Clone 0_2 and Clone 4430), which are heterozygous for a flower petal color mutation, will be used to develop an assay system for detecting mutagenic activity of airborne environmental chemicals. The contractor will further refine the assay system as to develop exposure techniques for exploratory work with known chemical mutagens. Known chemical mutagens will be used to develop dose-response curves to determine the relation sensitivity of clones 0_2 and 4430, as well as the lowest levels of exposure that can be detected.

MAJOR FINDINGS: (1) Safe handling procedures have been established for exposing the plants and for determining exposure levels using gas chromatography.

(2) Exposing Tradescantia Clones 0_2 and 4430 to ethyl methanesulfonate, ethylene dibromide and methyl sulfonic acid produced very high mutation responses. Good dose-response curves were obtained for both clones using 18, 54, and 88 ppm exposures.

(3) Although no significant differences were observed in their sensitivity to x-rays, Clone 4430 was found to be 10 to 20 times more sensitive to chemical mutagens than Clone 0_2. It is anticipated that Clone 4430 will respond to ethyl methanesulfonate at concentrations as low as 10 ppm.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract should provide a badly needed assay for mutagenic airborne environmental chemicals. It is extremely sensitive to ionizing radiation and the

exploratory work with selected chemical mutagens during this past year suggests that it may be able to detect concentrations as low as 10 ppm. This assay system should provide a meaningful assay for the production of gene mutations and relates to the Institute's program to develop sensitive assay systems to detect the mutagenic activity of environmental chemicals.

PROPOSED COURSE: The development of standard conditions of exposure will be continued and the effects of various environmental factors on the level of response determined. Emphasis will be placed on obtaining precise dose-response curves after treatment with different mutagens and to determine whether there is any evidence for thresholds.

THE DOW CHEMICAL COMPANY, MIDLAND, MICHIGAN (NIH-NIEHS-73-2102)

TITLE: "Study of the Effects of Environmental Components on Reproduction and Ontogenesis"

CONTRACTOR'S PROJECT DIRECTOR: B. A. Schwetz, D.V.M., Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: February 5, 1973

CURRENT ANNUAL LEVEL: $275,267

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the teratogenic potential in animals of environmental components when administered singly or in combination, (2) to determine species differences in response to teratogens and study the etiology of such differences and (3) to investigate the mechanism of action of teratogens and improve experimental testing and extrapolation to man.

METHODS EMPLOYED: In general, the screening approach outlined by the World Health Organization (Technical Report No. 364, 1967) will be followed. Specific modification and additions to the design will attempt to alleviate some of the problems encountered by private industry during the testing of potential therapeutic agents.

Pharmacokinetic differences in the dam, placenta and conceptus will be studied in an effort to discover the etiology of species differences and mechanisms of action for agents found to be teratogenic.

MAJOR FINDINGS: No results can be reported as the contract has just been initiated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The etiology of about 60% of human birth malformations is currently unknown. To date, most of the testing of environmental components for their capacity to alter the processes of mammalian development have been conducted by private industry which has motives and goals different from the basic scientist. As a result, too little is known concerning the teratogenic potential of chemical and physical factors in man's environment which acting either singly or in combination might account for an important fraction of human birth defects.

This contract will attempt to carefully evaluate the teratogenic potential of selected environmental factors. Chemicals will be administered both alone and in combination with other chemicals or factors which might alter the pharmacokinetics of the test agent and result in an alteration of teratogenic potential. Attempts will be made to understand teratogenic mechanisms which will allow for better predictive extrapolation from experimental animals to man.

PROPOSED COURSE: Agents are being selected for study on the basis of how widely they are used, how persistent they may be, and their relative likelihood of causing developmental problems.

DUKE UNIVERSITY, DURHAM, NORTH CAROLINA (NIH-NIEHS-72-2094)

TITLE: "A Study of Microwave-Neuron Interactions"

CONTRACTOR'S PROJECT DIRECTOR: William T. Joines, Ph.D.

PROJECT OFFICER (NIEHS): Robert H. Wyatt, Ph.D.

DATE CONTRACT INITIATED: June 1, 1972

CURRENT ANNUAL LEVEL: $9,947.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to determine how microwave energy within the 0.1 to 3.0 GHz frequency range interacts with the human nervous system. There are two basic tasks being undertaken on this project: (a) conduct studies aimed at determining the appropriate equivalent circuit of a neuron at microwave frequencies, and (b) develop instrumentation and measure induced transmembrane potential or any change in the firing pattern or activity of isolated nerve cells subjected to microwave irradiation.

MAJOR FINDINGS: This contract is now only in its first year. The efforts of the first 4 or 5 months concerned: (a) developing the experimental setup and performing functional test trials; (b) refining the setup further to isolate electrical interference noise sources; (c) performing theoretical calculations relating incident source power and power density within the sample neurons; and, (d) studying a new dipole theory of nerve excitation by L. Y. Wei in relation to the determination of a useful equivalent circuit of a neuron. In addition, further efforts have produced some extremely interesting results. The electrical activity of live neurons (the abdominal ganglion of Aplysia) was monitored while the neurons were being irradiated by 1.5 GHz microwave signals at various power levels, the object being to determine whether or not there is a direct interaction between the microwave and neurons. If such an interaction does occur, it should be detectable as some change in the normal firing pattern or electrical activity of the neurons. Obviously, a microwave-neuron interaction will occur if the power density is large enough to be thermally significant. Hence, it was necessary at an early stage of the project to establish an appropriate level of microwave power density to which the test neurons would be subjected. Because the results of this study should be relevant to the health and well-being of humans, it was decided that an appropriate level of power density would be the maximum which exists within the human brain when the skull is irradiated by 10 mW/cm^2 microwaves. Calculations on a multilayered sphere model of the human skull have shown that a maximum of about 20 mW/cm^3 occurs near the center of the brain (Joines and Speigel, 1973). Calculations show that the neurons would be subjected to a power density of 20 mW/cm^3 if the incident power from the microwave generator is 3.08 watts. Microwave interactions with isolated nerve cells have recently been obtained. The action potential of one neuron and the postsynaptic potential of another were monitored continuously while the microwave power was turned on and off. Experimental recordings show that there is a definite decrease in the firing rate as the microwave power level is increased. This appears to be a true

non-thermal, microwave-neuron interaction, because any increased heating caused by the microwaves would cause the firing rate of the neuron to increase, not decrease. With a neuron firing spontaneously with no stimulating current, 2 watts of generator microwave power stopped the neuron from firing; it started firing again immediately after the microwave power was shut off. The 1.5 GHz microwaves are undoubtedly influencing the electrical activity of the sample neuron. The question is: How? The sample neurons (plus surrounding seawater) absorbed about 0.05 watt of the 2 watts of microwave power, as determined by measuring the power reflected with the sample in place and then removed. Because the sample volume is about 1 cm^3, the absorbed power density is 50 mW/cm^3. This power density would cause a temperature rise of about 0.013°C/sec. which certainly would be considered thermally insignificant for the power-on times of this experiment. Besides, any thermal influences would be in the direction opposite from the experimental observations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A recent priority matter is the determination of the maximum safe exposure level of microwave energy for humans. This has been motivated significantly by the fact that safe exposure levels in the U.S. are 1000 greater than that set for Soviet-bloc nations. The level of the latter countries is largely based on indications of so-called non-thermal effects. There have been numerous effects of microwaves observed on neurological functioning: altered EEG patterns, modified motor activity, analepsis, induction of hallucinations, and the perception of "sound." Although biological effects of microwaves have been reported for several years, basic reasons and mechanisms underlying them remain poorly, if at all, understood, The question of the non-thermal influence of microwaves is the principal one concerning the biological effects of microwaves; it must be resolved before the degree of the hazard posed to man is determined. The contractor's accomplishments to date are significant in that they have produced what appears to be a direct microwave-neuron interaction; further experimentation is necessary before this is fully resolved.

PROPOSED COURSE: Further efforts will be made to identify the mechanism involved in the apparent microwave-neuron interaction. Careful study of the theoretical dipole model of the neural membrane and its interaction with microwaves will be made. If thermal effects are ruled out, there seems to be only three remaining possibilities: (1) the microwave signals are being rectified at the microelectrode-neuron junction, (2) microwave radiation from the stripline test fixture is being received and rectified at the preamplifier input (thus feeding a DC voltage back through the microelectrode to the neuron), and (3) the microwaves are interacting directly with the neuron. At present, contributions from (1) and (2) appear to be negligible. However, further measurements must be made before they can be ruled out altogether.

DUKE UNIVERSITY MEDICAL CENTER, Durham, N.C. (NIH-NIEHS-73-2101)

TITLE: "Study of Potential Long-Term Effects of Methotrexate Therapy"

CONTRACTOR'S PROJECT DIRECTOR: J. P. Tindall, Ph.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D.

DATE CONTRACT INITIATED: November 1, 1972

CURRENT ANNUAL LEVEL: $5,410 (six-month contract)

PROJECT DESCRIPTION

OBJECTIVES: (1) To obtain a roster of all psoriasis patients treated with methotrexate at the Duke University Dermatology Clinic prior to January 1, 1966. (2) To determine the current mortality status and major morbidity history of all such patients. (3) To prepare a roster of all psoriasis patients treated with methotrexate subsequent to January 1, 1966.

METHODS EMPLOYED: A review will be made of medical records of all persons treated for psoriasis at Duke University Dermatology Clinic during the period January 1, 1958, to December 31, 1971. Appropriate follow-up techniques will be used to determine current mortality status and major morbidity history of indicated patients.

MAJOR FINDINGS: The roster of patients treated prior to January 1, 1966, has been prepared. Follow-up studies are in progress with no major findings reported to date.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Definition of possible adverse long-term effects of this therapeutic drug has significance in regard to providing clues to the basic physiological mechanisms of carcinogenesis, mutagenesis, and teratogenesis.

PROPOSED COURSE: This contract will be terminated when the proposed studies have been completed.

GEORGIA INSTITUTE OF TECHNOLOGY, ATLANTA, GEORGIA (NIH-71-2374)

TITLE: "Study of Microwave Dosimetry"

CONTRACTOR'S PROJECT DIRECTOR: Harold Bassett and Gene K. Huddleston

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D.

DATE CONTRACT INITIATED: June 25, 1971

CURRENT ANNUAL LEVEL: $33,155.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of the contract is to develop techniques and detectors which can be used to measure the energy absorbed in a biological specimen placed either in the near or far microwave fields of frequencies ranging from 1 to 10 GHz.

MAJOR FINDINGS: Georgia Institute of Technology has developed the technology to fabricate microwave detectors using a pyroelectric crystal (triglycine sulfate). A probe has been built and supplied to us which has good sensitivity at 4-10 GHz. Below 4-10 GHz it is necessary to add an absorbing material to the crystal to provide adequate sensitivity. The probe must be placed in a modulated field due to the fact that the probe measures a rate of change in temperature rather than an absolute temperature. Attempts to modulate the probe electonically rather than the incoming field has proved unfruitful. Also the miniaturization of the probe from the 0.5 cm x 0.5 cm has encountered difficulty. The sensitivity of the probe decreases rapidly with decreasing size.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Instrumentation for the measurement of microwave energy is very limited. At present, there are no broad band detectors which are adequate for measuring energy absorption in biological material. With further development, this probe has the potential of becoming an accurate, sensitive detector for microwave fields of 1-10 GHz frequencies.

PROPOSED COURSE: The contractor will supply us with sensitive probes for free field measurements in the frequency range of 1-10 GHz. These probes will assist in the calibration of our variable frequency system. Also he expects to supply the Institute with a small implantible probe by the end of the contract period.

PUBLICATIONS

Huddleston, G. K. and Bassett, H. L.: Studies of microwave dosimetry. Presented at Symposium on Electromagnetic Hazards, Pollution and Environmental Quality, Purdue University, Lafayette, Indiana, May 8-9, 1972.

Huddleston, G. K. and Bassett, H. L.: Design of a microwave dosimeter. Presented at Microwave Dosimetry Workshop, Georgia Insitute of Technology, June 1-2, 1972. (Will be in the proceedings.)

GULF SOUTH RESEARCH INSTITUTE, NEW ORLEANS, LOUISIANA (NIH-NIEHS-72-2083)

TITLE: "Interaction of Metallic Salts In Vitro: Cell Biological, Analytical and Virological Aspects"

CONTRACT'S PROJECT DIRECTOR: B. H. Sweet, Ph. D.

PROJECT OFFICER (NIEHS): H. L. Falk, Ph. D.

ASSOCIATE PROJECT OFFICER (NIEHS): J. Spalding, Ph. D.

DATE CONTRACT INITIATED: 4-10-72

CURRENT ANNUAL LEVEL: $75,060

PROJECT DESCRIPTION

OBJECTIVES: To explore the interaction of important environmental trace metals in their effect on mammalian cells in tissue culture including their response to viral infection. This toxic effect has been described for certain trace metals in specific ratios and specific situations. A more general evaluation of the situation is desirable.

MAJOR FINDINGS: Acute toxicity was determined on the microscopic level for several inorganic salts of nickel, zinc, cadmium, copper, manganese and chromium in stationary cultures of human diploid cells over a seven day period. The lowest toxic level was determined by cytologic examination, by viable and total cell count and cellular protein determination. For some of these salts the toxicity was determined in actively growing cell cultures. Chronic toxicity over a 12 week period was studied for exposure to nickel, manganese, copper and cadmium salts. Cytogenetic studies and transformation were additional parameters of these studies. Flameless atomic absorption was the methodology of choice for the quantitation of the metal in the medium and the cell aggregate.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The human exposure to various trace metals is generally accepted to be a multiple metal event leaving a fair amount of speculation regarding the identity of the responsible metal in the etiology of the disease. It is also known that a protective effect is frequently observed by one metal over another regarding the biological system, so that in toxic environmental exposure an understanding of the interaction of trace metals is necessary. This contract will attempt to explore such interaction -- synergistic or antagonistic -- of trace metal on human cells in tissue culture to serve ultimately for studies in the intact animal.

PROPOSED COURSE: The study will focus next on the interaction of zinc, cadmium and nickel salts on human diploid cells in tissue culture as the first combination study. It will be followed by exploration of the effect of these salts on the susceptibility to viral infection and transformation. Other

trace metal combinations will be studied thereafter until most of the important toxic metals have been investigated in the combinations that would be meaningful from a knowledge of their appearance in the environment.

KRESGE HEARING INSTITUTE - UNIVERSITY OF MICHIGAN, ANN ARBOR, MICHIGAN
(NIH-NIEHS-72-2110)

TITLE: "Interaction of Noise and Ototoxic Drugs on Hearing Loss in Animals"

CONTRACTOR'S PROJECT DIRECTORS: Merle Lawrence, Ph.D. and Joseph Hawkins, Ph.D.

PROJECT OFFICER (NIEHS): Reginald O. Cook

DATE CONTRACT INITIATED: June 15, 1972

CURRENT ANNUAL LEVEL: $100,000

PROJECT DESCRIPTION

OBJECTIVES: To determine the extent and the nature of the interaction between noise and ototoxic drugs in experimental animals, and to study the biochemical mechanisms involved in such losses.

MAJOR FINDINGS: It has been determined that when nerve ending preparations of guinea pig cortex are incubated with ^{32}P, ^{32}P is incorporated into the following phospholipids - phosphatidylinositolphosphate, phosphatidylinositoldiphosphate, phosphatidic acid and phosphatidylinositol. Concentrations of 10^{-4}M kanamycin and neomycin and 10^{-3}M of streptomycin maximally increase labelling of the above phospholipids from ^{32}P in guinea pig cortex homogenate. Maximal stimulation of ^{32}P incorporation was 300% of controls for phosphatidylinositolphosphate. These concentrations are similar to serum concentrations of 20-50 μg/ml attained after clinical use of the antibiotics. Sucrose density gradient fractionation of homogenates indicated that this effect was most localized in nerve-ending fractions (synaptosomes).

10^{-4}M ethacrynic acid lowered ^{32}P-labelling of all lipids by approximately 70%, probably due to uncoupling of oxidative phosphorylation.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The burden from excessive noise in the general population is extensive and even more so among industrial workers. It now appears that certain drugs may greatly potentiate this noise damage at low levels of noise. This contract proposes to delineate suspected synergisms and investigate the biochemical mechanisms by which such damage occurs.

PROPOSED COURSE: Because preliminary experiments indicate that the aminoglycosides affect membrane components rather than the intrasynaptosomal ATP-pool, we feel that further studies with this system will be of considerable help in elucidating the ototoxic mechanisms of these drugs. γ-^{32}P-ATP will be used as precursor and synaptosomal subfractions will be investigated; the specificity of this drug action has to be assessed by comparison of the action of neomycin with the action of neomycin-fragments. In vivo studies of the incorporation of ^{32}P administered intraperitoneally into the lipids of control and drug treated cochleas will be conducted.

All studies will be conducted with guinea pigs divided into four groups of treatment: a) controls; b) drug; c) noise; d) drug and noise. Group d) may later be subdivided according to sequence of treatment to obtain maximum potentiation of the effects. Drugs studied will include neomycin, gentamicin, and ethacrynic acid.

The stria vascularis, the organ of Corti and Reissner's membrane will be examined separately, although the pilot studies will be on stria vascularis and spiral ligament.

Measurement of ATP levels and specific radioactivity of the γ - phosphate group of ATP as an indicator of the energy metabolism of the tissues will be accomplished via the phosphorylation in vivo of [^{3}H]2-deoxyglucose and separation of labelled metabolites by high voltage electrophoresis.

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
ALBUQUERQUE, NEW MEXICO
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Respiratory Tract Deposition Models"

CONTRACTOR'S PROJECT DIRECTOR: Otto G. Raabe, Ph.D.

PROJECT OFFICER (NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: November 11, 1971

CURRENT ANNUAL LEVEL: $103,119

PROJECT DESCRIPTION

OBJECTIVES: The major objectives of this research are:

(1) Construction of physical models of the respiratory tracts of man and several animal species;

(2) Measurement of the deposition of aerosols as small as 0.01 micron diameter in the models;

(3) Determination of deposition of selected particle sizes in animals and in man if practical;

(4) Development of a theoretical model of deposition in the lung based on the above results;

(5) Field evaluation of the physical-theoretical model.

MAJOR FINDINGS: About 75 negative casts of the lungs of several animal species and man have been produced by an in vivo replacement technique. Morphometric measurements are being made by hand and by stereoscopic radiography to classify every branch of the tracheobronchial tree according to length, diameter, branching angle, angle to gravity and any unusual characteristics. This information is used to generate a computer model of a representative lung. Deposition by diffusion, sedimentation or impaction can be calculated using the computer. Any improved formulation can be used in the computer program.

These results will be compared to experimental results on deposition in physical models and in the living animals corresponding to these models.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Improved respiratory tract deposition models are needed to develop insight into the manner in which the comparative morphology of the lung influences the deposition of inhaled particles in experimental animals and man. In particular, such a model would aid in establishing quantitative dose-response relationships in laboratory experiments and would effect more meaningful extrapolations to human exposure situations.

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, ALBUQUERQUE, NEW MEXICO (AT(29-2)-1013)

TITLE: "Toxic Effects of Commercial Detergent Products"

CONTRACTOR'S PROJECT DIRECTOR: D. A. Muggenburg, D.V.M.

PROJECT OFFICER (NIEHS): H. L. Falk, Ph. D.

ASSOCIATE PROJECT OFFICER (NIEHS): John Moore, D.V.M.

DATE CONTRACT INITIATED: May 4, 1972

CURRENT ANNUAL LEVEL: $100,506

PROJECT DESCRIPTION

OBJECTIVES: To develop realistic and reproducible bioassay procedures to evaluate the effects of ingestion of detergent products regarding early local and systemic, as well as long term, effects in young and adult dogs and pigs. To determine the effects of recommended remedial action following ingestion of detergent products or to develop and evaluate new remedial measures.

MAJOR FINDINGS: All commercial detergents tested showed a similar clinical pattern, which, however, varied considerably in severity. There was injury to the mucous membranes of the mouth, pharynx and esophagus, to the wall of the stomach, to the larynx, to the lungs due to inhalation of detergent or aspiration of vomitus and damage to the kidneys. Results were similar for the dog and the pig, but the young dog was more sensitive than the mature dog, while the difference in sensitivity was not observed in the pigs.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of the eutrophication problem in inland waters, the use of polyphosphates in detergent formulations has been reduced or discontinued leading to greater utilization of sodium carbonate and metasilicate which are harmless to the environment, but because of their high alkalinity are much more corrosive and harmful to the young child that always must explore interesting powders in the kitchen and may swallow some of the material. The ingestion of detergents -- about 4000 cases a year in the U.S.A. and Canada -- by young children will be far more disastrous when the detergent builder is highly alkaline carbonate or metasilicate compared to polyphosphate.

PROPOSED COURSE: The contract will be terminated when the program is completed and the results will be published as soon as possible.

MIDWEST RESEARCH INSTITUTE, KANSAS CITY, MISSOURI (NIH-NIEHS-72-2090)

TITLE: "An Appraisal of Environmental Exposure to Heavy Metals"

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, Ph. D.

PROJECT OFFICER (NIEHS): H. L. Falk, Ph. D.

ASSOCIATE PROJECT OFFICER (NIEHS): Warren T. Piver, Ph. D.

DATE CONTRACT INITIATED: April 17, 1972

CURRENT ANNUAL LEVEL: $48,640

PROJECT DESCRIPTION

OBJECTIVES: To explore environmental contamination with specific metals which may be hazardous to health. To search the literature and other reports and to seek expert opinions from industry in order to compile these data on potential hazards of specific metals. Special emphasis is placed on determination of the physical parameters and chemical properties, valency, reactivity, etc., as the metal compound makes contact with man. The information is to be put together in a document on each of the metals of specific interest.

MAJOR FINDINGS: The first document dealt with the metal osmium. It contained data on the reactivity of osmium compounds in the environment. The search for information itself was very informative considering that a lot of information is being gathered by many scientists in many laboratories, but with regard to toxic trace metals the nature of the compound or complex is usually not determined which is a function of the level of the metal in the environment and the lack of specificity of the analytical method used.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals or different forms of metals, or their complexes. The information will determine whether research on the health effects of the compound in question should be undertaken and in which direction it should go.

PROPOSED COURSE: The information is being gathered for a number of metals of importance from the toxicologic viewpoint. So far, only one metal has been studied well enough to be reported. The other metals will have to follow.

MIDWEST RESEARCH LABORATORIES, KANSAS CITY, MISSOURI (NIEHS-72-2084)

TITLE: "Long-Range Toxicologic Effect of Metal-Containing Dithiocarbamate Fungicides"

CONTRACTOR'S PROJECT DIRECTOR: C. C. Lee, Ph.D.

PROJECT OFFICER (NIEHS): E. O. Oswald, Ph.D.

DATE CONTRACT INITIATED: February 1, 1972

CURRENT ANNUAL LEVEL: $134,207

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the potential toxicologic effects of selected metal-containing dithiocarbamate fungicides and major degradation products, specifically: (a) by investigating the stability of ferbam, maneb and zineb in commercial formulations upon ordinary storage conditions and on seeds of crops and/or growing plants; longterm toxicologic effects will be studied on a total of four compounds, the parents and major decomposition products; (b) by studying the rate of absorption, retention, storage, blood levels, excretion, and metabolic products of the selected compounds; (c) by elucidating the toxic effects on the fertility and reproductive performance in male and female rats, the teratological effects in rats and mice, and the prenatal and postnatal effects of the selected compounds in rats; and (d) by determining the acute, subacute, and chronic toxicities of the selected compounds.

METHODS EMPLOYED: Conventional chromatographic procedures, U.V., visible, I.R., spectroscopy, NMR and mass spectrometry, histological techniques, and radiotracer analyses.

MAJOR FINDINGS: Using ferbam, maneb and zineb which had been earlier analyzed for cobalt, nickel, lead, thallium, selenium, iron, manganese and zinc, it was concluded that the LD_{50} of ferbam, maneb and zineb in rats approximated 4.6g/Kg, > 12.6g/Kg, and > 10.0g/Kg, respectively. Ferbam caused hyperactivity and salivation; maneb and zineb caused hair loss in some animals. Ferbam dissolved in acetonitrile, or sprayed on growing bean seedlings, was found to yield significant quantities (17-34%) of thiram. Subacute feeding studies using male Charles River CD rats indicate that 1.2% ferbam in feed was lethal after two weeks, 0.25% was toxic, and 0.05% was tolerated.

Increasing intake of active ferbam in rats in quantities from 22.9 mg/Kg/day to 109.1 mg/Kg/day produced a significant increase in reticulocytes, from 0.93 ± 0.10% to 1.78 ± 0.09%, respectively. In addition, with the above specified intake of ferbam, blood urea nitrogen (BUN) increased from 14.9 ± 0.6 mg% to 19.8 ± 1.4 mg%, respectively.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These investigations will establish the effect and nature of the acute, subacute and chronic toxicities of the selected dithiocarbamate fungicides and their most

significant degradation products. This information will serve to form a rational basis for evaluation of the potential health hazards produced by the use and exposure of these fungicides.

PROPOSED COURSE: To complete the specified objectives of the contract in relationship to the metabolism and the potential toxic effects of specified metal containing dithiocarbamate fungicides and major degradation products.

Biology Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee

TITLE: "Environmental Mutagen Information Center"

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTE: National Cancer Institute

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: $102,000

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to create, maintain and perpetuate a data base of chemical mutagenesis information.

MAJOR ACCOMPLISHMENTS: EMIC's data file contains 8,500 bibliography entries. Of these, approximately 3,000 citations have been indexed with respect to agent and organism. CA Registry numbers for those compounds listed in the above agent keyword are now being added. The programming and handling of data extracted from the literature have been completed and can be presented in a tabular form. The EMIC/GRAS Literature Review was completed. The final document contained over 600 pages of text, references, tabular abstracts and other indexes. The Center processed 152 requests during calendar year 1972 for information on particular subjects. The requests arrived at the Center at a rate of 2-3 week and came from many locations throughout the United States as well as several foreign countries.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (950). EMIC has helped several national commissions in collecting and extracting the data from the literature. For the Institute and especially the Mutagenesis Branch, EMIC saves more than one man-year of time for the personnel in the Branch in supplying the literature references in special areas.

PROPOSED COURSE: EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objects as well as indexes for agents and selected title keywords. As great a portion as possible of the previous bibliographic

entrees will be keyworded with respect to agent, test organism, and test object. EMIC will continue to answer selected requests for special information from the scientific, educational and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in conjunction with timely state of art reports on subjects of current scientific interest. The staff of EMIC is presently working on (a) effect of chemicals on mammalian germ cells or germ cell tissue, (b) mutagenicity of polycyclic hydrocarbons, and (c) mutagenicity of selected carcinogenic compounds.

PUBLICATIONS

Wassom, J. S.: The literature of chemical mutagenesis. In Hollaender, A. (Ed.): Chemical Mutagens, Volume III, Plenum Press (In Press).

Wassom, J. S.: The storage and retrieval of mutagenic information (Abstract of a paper delivered to the First Annual Collaborative Carcinogenesis Conference, San Antonio, Texas, Oct. 2-4, 1972, Information and Resource Segment Publication, Page 23).

Newar, M. M., Malling, H. V., Wassom, J. S. and von Halle, E. S.: A review of the mutagenic potential of a selected number of food additives. EMIC-ORNL Technical Repo-t #1 (Teratogenicity Section), pp. 1-269, 1973.

Brown, M., von Halle, E. S., Malling, H. V. and Wassom, J. S.: A review of the potential teratogenicity of a selected number of food additives. EMIC-ORNL Technical Report #1 (Teratogenicity Section), pp. 1-179, 1973.

Wassom, J. S., von Halle, E. S., Shelby, M. D., Miller, I. R., Newar, M. M. and Brown, M. M.: A survey of the 1971 literature of chemical mutagenesis. EMIC-ORNL Technical Report #3, 1973 (In Press).

SOUTHWEST RESEARCH INSTITUTE, SAN ANTONIO, TEXAS (NIH-72-2035)

TITLE: "The Interaction of Specific Air Pollutants with Lung Lipids"

CONTRACTOR'S PROJECT DIRECTOR: R. Bollinger, Ph.D.

PROJECT OFFICER (NIEHS): Phillip W. Albro, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $174,239

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the effects and mechanisms of interaction of specified gaseous compounds with lung membrane lipids *in vivo* and *in vitro*; to clarify the chemistry and biochemistry of such interactions; to attempt to correlate the structural changes observed on a chemical level with any significant functional changes; and to examine possible reversal, protective or preventative approaches.

METHODS EMPLOYED: The basic test animal is the rabbit; *in vitro* systems include lung slices, lung mitochondria and cells from lung washes. Various conventional chromatographic procedures, NMR and IR spectroscopy and histological techniques are used. ESR is used to detect alterations in membrane components *in situ*.

MAJOR FINDINGS: NO_2 and NO reacted with unsaturated lung lipids and HCHO reacted with free amino groups of lung phospholipids and proteins *in vivo* and *in vitro*. Some of the Freons stimulated lung lipolytic activity, while lipolytic activity in lung homogenates was decreased by o-phenylphenol and morpholinium, and possibly by SO_2.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project studies in detail a specialized aspect of the general chemical responsivity of the lung to commonly inhaled substances, and thus should ultimately aid in predicting responsivity to other substances of interest.

PROPOSED COURSE: The second year's effort will be to verify, quantitate and extend the *in vitro* findings to pollutant exposed animals.

STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIFORNIA (NIH-NIEHS-72-2085)

TITLE: "Alteration of the Blood-Brain Barrier by Toxicants"

CONTRACTOR'S PROJECT DIRECTOR: C. Mitoma, Ph.D.

PROJECT OFFICER (NIEHS): Robert L. Dixon, Ph.D.

DATE CONTRACT INITIATED: April 17, 1972

CURRENT ANNUAL LEVEL: $131,569

PROJECT DESCRIPTION

OBJECTIVE: To determine the effect of environmental toxicants on the blood-brain barrier (BBB).

METHODS EMPLOYED: The adopted procedure consists of injecting a mixture of a labeled test solute and tritiated water intravenously into the mouse and examining the radioactivity content of the brain 20 minutes later. Three test solutes--α-methyl-D-glucoside (α-MG), 2-aminoisobutyric acid (AIBA) and Na^+--were routinely used. The extent of uptake of a solute is expressed as a percentage of the uptake of tritiated water.

MAJOR FINDINGS: The brain uptake indices of the solutes are approximately 3 for α-MG, 40 for AIBA and 16 for Na^+.

Two chemical agents known to cause osmotic imbalance, namely, glycerol and urea, increased the brain uptake index of α-MG and Na^+. The effect on the BBB of chronic exposure of mice to three to five months to low levels of phenobarbital, DDT, lead chloride, methylmercury, paraoxon and ethanol are being investigated. An acute dose of none of these agents altered the BBB. The data obtained so far indicate that exposure to lead chloride and methylmercury may have lowered the brain uptake of α-MG.

The ultrastructural studies during the first two quarters centered around technique development. Control tissues fixed "en bloc" or through systemic perfusion were evaluated. Consistently excellent preservation has now been obtained through systemic perfusion with Karnovsky's fixative.

Animals have been treated with urea, which is known to interrupt the barrier. Both electron and light microscope "tracers" have been utilized to visualize the points of defect. Success has been achieved in interrupting the barrier, and data collection will continue during the next quarter.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The blood brain barrier protects the central nervous system and preserves its unique milieu. Recognition of environmental toxicants which might alter this protective barrier and result in toxic effects to the nervous system is of obvious importance.

PROPOSED COURSE: Because of competing demands for resources, this contract is being discontinued.

UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE, ROCHESTER, NEW YORK
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Uranium Mining - Health Hazards Associated with Radon Daughters"

CONTRACTOR'S PROJECT DIRECTOR: William F. Bale, M.D.

PROJECT OFFICER (NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: June 30, 1968

CURRENT ANNUAL LEVEL: $119,000

PROJECT DESCRIPTION

OBJECTIVES: In five separate but closely interrelated projects, senior scientists have and/or are conducting the following studies:

Project A - Dr. Donald Morken has exposed dogs and rats to radon and radon daughters on room air nuclei. The magnitude and intensity of exposure versus early histopathological effects are important initially.

Project B-1 - Dr. John Hursh has studied the bone and tissue distribution of ^{210}Pb as well as excretion of ^{210}Pb and ^{210}Po using dogs exposed in Project A.

Project B-2 - Dr. Hursh has measured the transport time of ^{212}Pb from human lungs.

Project C - Dr. Thomas Mercer has characterized the radon daughter carrying aerosols used in the studies by Morken and Hursh.

Project D - Dr. R. W. Helmkamp and Dr. W. F. Bale have developed new techniques for the measurement of ^{210}Po and ^{210}Pb in urine.

MAJOR FINDINGS: Projects B-1, B-2, C and D have all been completed. Publications based on this work are listed below. Only Project A remains to be completed. The major findings for Project A are as follows:

1. Lung Histopathology - A preliminary pathology report for a total of 36 dogs sacrificed at exposure levels of 200, 400, 800, 1600, 3000 and 10,000 WLM at zero, one, two and three years post exposure is available. Age control and chamber control dogs were also included. No cancer is evident in any dog. This does not mean that cancer would not have occurred later.

   The microscopic lesions observed, even at the highest dose levels, are subtle, variable, very small spatially, widely separated and involve only a small fraction of the lung substance.

2. Radiolead dog bones - Femur and rib samples have been taken from all dogs sacrificed. These samples have so far been digested and await radiolead analysis.

3. Determination of dose to dog lungs - On the basis of sacrifice of 11 dogs immediately after exposure and subsequently counting the RaB and RaC activity, the average dose in rads per WLM exposure was determined. The average dose rate to the whole lung was 0.17 rad/WLM. Individual dose rates varied from 0.08 - 0.79 rad/WLM. Clearance rates in the trachea and bifurcation were very high but clearance in the bronchial tree and alveolar region due to ciliary activity was apparently not significant. The dose rate to the upper respiratory tract was about 30 times the whole lung dose. For the dogs, the alveolar doses probably ranged from 30-1700 rads while the bronchial doses may have ranged from 1,000-50,000 rads.

4. Rat exposures - Exposure of 100 normal (not specific pathogen free) and 100 specific pathogen free rats is nearing completion. The protocol for these experiments was similar to French experiments in which nearly 100% cancer incidence was obtained about 10 months post exposure. Results of the current experiments will be available in the near future.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is an integral part of research directed toward the resolution of the uranium miner-lung cancer problem. This project should yield useful information on induction of lung cancer and on heavy metal metabolism.

PROPOSED COURSE: Projects B-1, B-2, C, and D have been completed. All experimental work on project A is to be completed during FY 1973. Work during FY 74 will involve analysis of final data and preparation of final reports.

PUBLICATIONS

Hursh, J. B. and Mercer, T. T.: Measurement of ^{212}Pb loss rate from human lungs. Journal of Applied Physiology 28:268-274, 1970.

Mercer, T. T.: The deposition of unattached decay product atoms in an impaction stage. Health Physics 17:259 (1969).

Blair, H. A.: Dose-time relations for induction of lung cancer in uranium miners. Reprint from symposium on "Radiation-Induced Cancer." International Atomic Energy Agency, Vienna (1969).

Hursh, J. B.: Retention of ^{210}Pb in beagle dogs. Health Physics. (In Press).

Helmkamp, R. W., Hrynyszyn, V., Contreras, M., Bale, W. F.: Development of a method for measuring low levels of ^{210}Po in human urine. (In Press).

The University of Texas at Austin (NIH-NIEHS-70-2288)
Austin, Texas

TITLE: "Detection of Human Somatic Mutation"

CONTRACTOR'S PROJECT DIRECTOR: H. Eldon Sutton, Ph.D.

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

DATE CONTRACT INITIATED: June 30, 1970

CURRENT ANNUAL LEVEL: $32,444

PROJECT DESCRIPTION

OBJECTIVES: The contract objective is to develop methods of monitoring mutagenesis in human population, with initial studies to be done on a system involving glucose-6-phosphate dehydrogenase variants in white blood cells and persistence of fetal hemoglobin in red blood cells.

MAJOR FINDINGS: (1) Glucose-6-phosphate dehydrogenase (G6PD): The system is based on the observation that the genetic variants of human G6PD are capable of acting on artificial substrates such as 2-deoxyglucose-6-phosphate. It was found that similar changes could occur in the normal G6PD due to environmental agents. At the present time, reliable methods based on G6PD variants may be difficult to develop. Therefore, the G6PD system is not being pursued any further. (2) Fetal hemoglobin in red blood cells: The system is based on the observation that human mutations are known that result in persistence of fetal hemoglobin in red blood cells. It was found that the presence of red blood cells which appear to have fetal hemoglobin occurred with a frequency below 37 per 10^6 red blood cells among 7 tested individuals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds in the environment are known to be mutagenic. An increase in the mutation rate in the human population would be potentially detrimental; however, there is no test system available to monitor the human population for point mutations. Development of such systems would make it possible to monitor populations of high risk, e.g., individual administered certain drugs or workers in the pesticide industry.

The proposed research relates to our own in-house research, where we are attempting to develop a mutation system in mice based on detection of the frequency of red blood cells containing fetal hemoglobin in laboratory mammals after mutagenic treatment.

PROPOSED COURSE: This particular contract will be terminated by the end of FY 1973.

The University of Wisconsin (NIH-NIEHS-72-2095)
Madison, Wisconsin

TITLE: "Mutation Studies with Cultured Human Cells"

CONTRACTOR'S PROJECT DIRECTOR: Robert DeMars, Ph.D.

PROJECT OFFICER (NIEHS): Frederick J. de Serres, Ph.D.

DATE CONTRACT INITIATED: June 15, 1972

CURRENT ANNUAL LEVEL: $82,000

PROJECT DESCRIPTION

OBJECTIVES: Develop a mutational assay system which will provide a means of monitoring mutations among somatic cells of humans. This system shall utilize the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus in diploid, human fibroblasts.

METHODS EMPLOYED: The contractor is expected to isolate mutants at the azaguanine phosphoribosyltransferase (AGPRT) locus by looking for mutants resistant to 8-azaguanine. This work is also to entail a study of both spontaneous and induced forward-mutation rates at this locus.

MAJOR FINDINGS: Studies are in progress to develop assays to detect gene mutations both at the HGPRT locus (located on the x chromosome) and the AGPRT locus on chromosome 16. Mutants at the former locus are 8-azaguanine resistant and at the latter locus 2,6 diaminopurine resistant. Studies with cultures from different human subjects have shown that the average incidence of spontaneous mutants is about 5×10^{-6}. Mutants have also been shown to increase after treatment with N-methyl-N'-nitrosoguanidine (MNNG) and x-rays. Pedigree analysis has made possible the identification of human subjects heterozygous at the AGPRT locus, and fibroblast cultures from these individuals have been used to obtain 2,6 diaminopurine-resistant mutants.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to our attempts to develop assays to screen environmental chemicals for mutagenic activity (e.g., production of gene mutations). The development of generalized methodology will make it possible to study spontaneous and induced forward-mutation at the HGPRT locus in any individual in the population. Individuals of portions of the population at high risk can also be examined to determine whether the "spontaneous" frequency of HGPRT mutants is unusually high. Given cell lines can also be used to screen chemicals for mutagenic activity. Humans heterozygous at the AGPRT locus can usually be identified by pedigree analysis, and fibroblast cultures from these individuals can be used for analyses at either locus.

PROPOSED COURSE: Biochemical characterization of enzymes from mutant cells is in progress and will be continued along several lines of investigation to insure that mutants at both loci result from alteration of the gene and not

some other mechanism. The contractor will also study spontaneous mutation in fibroblasts derived from at least 10 individuals to study spontaneous mutation at the HGPRT locus and to develop experimental conditions and cell lines with low spontaneous forward-mutation rates. In addition, some work will be started to explore the utilization of this system for genetic analysis of important environmental chemicals.

U.S. FORESTRY SERVICE, RESEARCH TRIANGLE PARK, NORTH CAROLINA (NIEHS-72-2206)

TITLE: "Influence of Microorganisms and Other Soil Factors on Degradation of Certain Insecticides"

CONTRACTOR'S PROJECT DIRECTOR: Felton L. Hastings, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: August 24, 1971

CURRENT ANNUAL LEVEL: $25,714.00

PROJECT DESCRIPTION

OBJECTIVES: To study the influence of microorganisms and other soil factors on the degradation of certain insecticides, the insectides of particular interest being Mirex, Kepone, DDVP, Disulfoton, Temik and Dursban.

METHODS EMPLOYED: Special extraction procedures were developed for each of the insecticides studied. Gas and thin-layer chromatography were used in the analysis of the extracts, and radiotracer techniques were used to detect metabolism and to locate the metabolites.

MAJOR FINDINGS: The first year's work was concentrated on Mirex, the current work was concentrated on Temik. No breakdown of Mirex was detected after 6 months in nine aerobic or in four anerobic soils. Further, degradation of Mirex was not detected after 2-week incubations with thirteen individual bacterial species in synthetic liquid culture. In addition, Mirex was shown to have little or no effect on soil microorganisms, even when relatively high concentrations of the compound were added to the soil. Leaching studies have shown Mirex to remain in the soil indefinitely.

Temik was shown to be metabolized primarily to the sulfoxide with lesser amounts of the sulfone, oxime sulfoxide, oxime sulfone, nitrile sulfoxide, and nitrile sulfone also being produced. After 7 days incubation time the ratios of parent compound and metabolites are as follows: Temik 55%, sulfoxide 43%, and other metabolites 2%.

PROPOSED COURSE: The work initiated on Temik will be completed during the remainder of the contract year. The contract will be terminated at the end of the current year.

OFFICE OF THE SCIENTIFIC DIRECTOR

OFFICE OF THE SCIENTIFIC DIRECTOR
Summary Statement

The Intramural Research Program of the National Institute of Environmental Health Sciences is designed to serve two primary functions: first, to provide basic biomedical research information specifically related to the ultimate effects of the long-term, low-level exposure of environmental chemicals and factors on the human population. Of particular importance are those effects which occur after a delayed period of time. This information is necessary to provide health-oriented agencies and personnel the best information as to the cause and mechanism of diseases due to environmental origins as well as to provide regulatory agencies the information necessary to promulgate proper regulatory decisions. Second, and in addition to this ongoing research program, there is a predictably unpredictable need to respond to current acute problems of environmental agents immediately and adversely affecting human health. This is a broad, important mission and obviously, to be totally effective, NIEHS would require an intramural staff approximately three times that which is presently available. It has become necessary, therefore, to sharply focus those major efforts of the Intramural Research Program into areas of recognized needs consistent with available resources.

Classic environmental health research has been effect-oriented. A typical experiment involved the administration of a compound to an animal, a holding period, and then an autopsy of the animal to assess, based on tissue damages, what -- if any -- deleterious effects occurred. While this sort of experiment is still absolutely necessary, it is apparent that too little attention has been paid to what happened between the administration of the compound to the animal and the time the active agent reaches the site of action, interacts with that site, and initiates the eventual toxicological response. This entire process can best be described as pharmacological disposition and includes the sequential and parallel steps of absorption, distribution and storage, metabolism, excretion, and penetration of tissue and cellular barriers. In order to effectively extrapolate toxicity data from laboratory animals to man, it will be necessary to understand the pharmacological disposition of chemical agents and classes of chemical agents in the various laboratory animal models and eventually in man. It is clear, therefore, that this is one major area of importance in our intramural reseach program.

Effect-oriented research, of course, is still necessary. The research program at NIEHS is influenced by three primary factors: First, the perceived national needs with respect to the public health; second, the in-house scientific competence; and third, the existing physical facilities. Consistent with these three factors, the areas of major importance at NIEHS are: 1) the effects on genetic material -- that is, research on mutagenicity and mutagenicity testing; 2) the effects on reproductive processes and the perinatal period including,

quite obviously, teratology; 3) the effects upon the lung; and 4) the effects on biological material of such ubiquitous physical factors as noise and non-ionizing radiation.

These areas -- pharmacological disposition, mutagenesis, reproduction and teratology, and lung and physical factors -- may be considered to be one coordinate in a two-dimensional matrix. The other coordinate must be the general types and classes of compounds to be studied. Again, it is important to look at the criteria by which compounds are selected that are likely to be of public health importance. Of these criteria, perhaps three are most important: 1) likely to be produced in a large quantity; 2) likely to be persistent in the environment; and 3) likely to be toxic to biological material. For instance, the PCBs are produced in very large quantities, are likely to be very persistent, and may or may not be likely to be toxic; but, because of the first two factors, they are of great potential interest. The chlorinated dioxins, on the other hand, are not produced in very large quantities, are only moderately persistent, but are extremely toxic and therefore are of major importance.

In considering which compounds of the various classes to study, one must emphasize that for major classes of chemicals and toxicological effects, studies on the mechanism of action present an opportunity ultimately to maximize scientific effort, time and money. If a study can elucidate the mechanism of action of one compound, it is often possible to generalize these other closely related compounds. This can shorten drastically the time and effort needed to assess the safety or toxicity of other related compounds.

Another major important consideration is that of test methodology and test validation. What is the best test procedure? What is the most effective and most efficient way of looking for the toxic effects under consideration? What is the significance of a positive or negative response on each test? What is the result in terms of relevance for human health? Our mission does not preclude us from direct toxicity testing. However, at the present time, this is such an expensive and time consuming process that it should be done only on unusual occasions and with very critical compounds.

One other aspect that must be considered through the entire fabric of the intramural research activity is the problem of synergistically toxic interactions. Some of the best scientists in this field are convinced that major environmental health threats will not come from a single compound alone but will come when two relatively non-toxic compounds are present together and cause unexpected synergistic toxicity. This has been amply demonstrated in the field of therapeutic drugs, but little research has yet been done in the area of environmental pollutants.

It follows then from this discussion that the following research areas have branch status in the intramural research program at NIEHS: 1) Pharmacology and Toxicology -- which includes pharmacological disposition and studies on the lung; 2) Pathologic-Physiology -- which includes studies of reproductive toxicity and teratology; and 3) Mutagenesis. Research programs are, in addition, an integral part of the other three branches -- Animal Science and Technology Branch, Biometry Branch, and Research Services Branch -- which also have significant service responsibilities.

The summaries of each individual Branch adequately reflect the very significant research progress made during the past twelve months, and these shall not be recapitulated here.

ANIMAL SCIENCE AND TECHNOLOGY BRANCH

ANIMAL SCIENCE AND TECHNOLOGY BRANCH
Summary Statement

General

Review of Branch activities during FY 1973 reveal that two events were dominant: a) Branch programs initiated in previous years approached their projected level of operational effectiveness. b) Institute reorganization enlarged the scope of Branch mission by including analytical and synthetic chemistry personnel and programs as well as the histology and electron microscopy service laboratory in its structure.

The Branch objectives which reflect reorganization within the Institute are fourfold:

1. Program and coordinate experimental animal procurement, housing and utilization within the Institute.

2. Maintain collaborative support laboratories in the disciplines of Chemistry, Microbiology, Pathology, Histology and Electron Microscopy, and Veterinary Medicine.

3. Develop, refine and advise on the appropriate animal models for use in specific NIEHS research programs.

4. Plan and conduct research appropriate to the function of Branch laboratories.

The immediate goal of the Branch is to integrate recently assimilated diverse groups into a structure that directly focuses on Branch objectives. Due to limited resources, some current areas of research will be curtailed. Resources made available as a result of this action will be directed to areas of defined Institute priority. By developing and interrelating research within the Branch, and with other Branches, collaborative programs which are the most effective use of finite resources are developed.

Support Activities

Most of the Institute's research animals are housed within Building 15, a structure which rigidly controls their physical and biological environment. The average daily census approximates 16,000 animals. To accommodate the large number of adult rabbits used in reproduction and ontogenesis studies, a screened, roofed structure capable of holding 300 rabbits during early quarantine was constructed in September 1972.

Health surveillance activities which monitor an animal's suitability for research through definition of pathogen burden and sanitation level in a controlled environment continue to be a major effort. Random bred rats and mice of very high quality are used by the Institute. Problems in procurement of healthy inbred mice and guinea pigs in adequate numbers necessitated production and issuance of these animals in NIEHS colonies. Efforts to locate suitable commercial sources continue. Locating reliable sources will allow for the disbanding of NIEHS colonies which are demanding on limited space and personnel.

A revised cooperative training program with the Durham New Careers Program was completed. The program successfully trained 8 individuals selected from financially, educationally and culturally deprived backgrounds. Where the dropout rate in previous programs was quite disappointing, all trainees completed the latest program and were placed in entry level positions within the Animal Husbandry Section. Extra effort during the interview process apparently insured selection of individuals with self-motivation and education potential.

In-service training routinely continues as typified by repetitive classes in basic laboratory animal techniques. A course in general biology, initiated as part of the New Careers training program, is to be offered to other animal care personnel and interested technicians throughout the Institute.

The Cooperative Training Program, whereby 3 students majoring in chemistry from N. C. A & T University complete part of their education by on-the-job experience within NIEHS laboratories, has been mutally rewarding to the students and NIEHS chemists.

Collaborative and service laboratory activities are requested daily. About one third of the clinical pathology and microbiology laboratory effort are devoted to processing samples submitted by Institute scientists. Histology and electron microscopy services as well as specific requests for animal matings, dosing and other techniques exceeds our capacity.

Characterization of the biology of the captive Virginia opossum continued. Efforts were primarily directed toward developing a hematology and clinical chemistry profile, determining factors which account for the captive animals susceptibility to septicemias, and evaluation of reproductive performance. Emphasis on defining the biology of the opossum will be curtailed at the end of the FY in order to devote resources to problems of higher priority within the Institute.

Research Activities

Research on the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin was expanded and intensified. The toxic and lethal effects which occur under various dose regimens were defined. Pathologic evaluation indicated that lymphoid organs, especially the thymus, were consistently affected over a wide spectrum of doses in all species examined. These findings correlate with observed effect on immunocompetence. The cellular immune response is suppressed in guinea pigs and mice whereas the humoral antibody response measured in the guinea pig was not affected. Although liver effects are consistently seen, the degree of hepatic

involvement produced at a given dose was quite variable between species. Studies as to the effects of TCDD on the clotting mechanism are also in progress. Further experiments as to the postnatal effects of maternal TCDD exposure confirmed that some kidney effects observed prenatally are not reversible delays in organ maturation. The pathogenesis of this apparent hydronephrotic, not cystic, abnormality is continuing to be investigated. Additional studies using other chlorinated dioxins as well as dibenzofurans are planned.

Procedures which assess the effects of environmental chemicals on host resistance are being developed. Test methods used in assessing TCDD effects on immunocompetence form the core of procedures to be employed. The immunosuppressive effects of other environmental chemicals such as dibenzofurans and methyl mercury will be evaluated.

Previous studies in this laboratory identified hycanthone methanesulfonate as a potent teratogen in mice. Recent investigations extend the embryolethal and teratogenic finding to rats. Further studies with other structural analogs are planned primarily through contract activities.

Research on polychlorinated biphenyls was concentrated in several areas. Development of analytical methodology to quantify total PCB when isomeric configuration is unknown is in progress. Studies on the pathological and immunologic effects of Aroclor 1221, 1242 and 1254 in rabbits were undertaken. Severity of pathological effects increased as the chlorine content of the PCB increased. Experiments on the effects of long-term exposure and subsequent starvation in rats are partially complete.

Specific environmental agents under investigation within the Chemistry Section are such man made products as: (1) Chlorinated Hydrocarbons including (a) DDT and its metabolites; (b) Dieldrin, Aldrin, and similar polycyclodienes and metabolites; and (c) Polychlorinated Biphenyls (PCBS), chlorinated phenols, and metabolies; (2) metal containing fungicides including dithiocarbamates; and (3) Plasticizers including phthalate esters with specific reference to Di(2-ethylhexyl) phthalate. Environmental agents presently under investigation by the Chemistry Section include: (1) Heavy metals with specific reference to (a) organic and inorganic mercury; (b) cadmium; and (c) arsenic in the forms of arsenate and arsenite; (2) Rare earth metals including europium and praseodymium in respect to enhancement of low level analytical quantitation and enhancement of NMR characterization and prediction capabilities; (3) Amines, nitrosamines, and amino acids; and (4) Lipid soluble natural products including: (a) long chain aliphatic hydrocarbons with specific reference to alkanes and branched hydrocarbons such as phytane; (b) aromatic polynuclear hydrocarbons with specific reference to interaction of aromatic carcinogens in biological systems as studied by ^{13}C NMR; (c) allyl and propenyl benzenes of foods and essential oils; and (d) prostaglandins.

Serial No.: NIEHS-AST-001-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Health Surveillance of NIEHS Laboratory Animals"

PREVIOUS SERIAL NUMBER: NIEHS-AST-001

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.&A.H., Ph.D. and Julius E. Thigpen, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	3.55
Professional:	1.15
Other:	2.40

PROJECT DESCRIPTION

OBJECTIVES: To monitor incoming, resident, and alternative source laboratory animals for spontaneous infectious diseases, parasites and other factors that may compromise the animals' utilization in research.

METHODS EMPLOYED: Animals from new sources, and shipments from current sources, are selectively examined for ectoparasites, endoparasites, respiratory and enteric pathogens. Rats and rabbits are retained for appropriate periods of time before being issued for research. An attempt is made to necropsy all animals that die spontaneously. Routine quality control procedures monitor the effectiveness of sanitation procedures associated with food, bedding, clothing, cages, room or other items which research animals may contact. Standard microbiological procedures are used to detect bacteria, pplo, fungi and viruses. (Specific regimens employed for each species were previously described (FY 72 Annual Report).)

MAJOR FINDINGS: CD rats have been free of parasites and respiratory or enteric pathogens.

Aside from occasional deaths resulting from malocclusion or fighting, randombred and NIEHS inbred colonies of mice are disease-free. Serological titers to Sendai and GDVII viruses are consistently found. One inbred mouse strain ($C_3D_2F_1$) has been retained in isolation because of mite infestation. Disease-free inbred mice are not available for procurement.

Guinea pigs are no longer purchased but are produced by an NIEHS breeding colony. The primary reasons for this are three-fold: (1) need for date bred pregnant sows, (2) need for neonates free of subclinical ear infection, and (3) routine receipt of guinea pigs with respiratory disease. Respiratory infections, possibly due to Bordetella bronchiseptica, are infrequently encountered as are maternal deaths from "pregnancy toxemia". Hair loss in dams and offspring has been relatively common. This seems to be a social vice due to hair chewing by cage mates because hair regrows while the animal is caged individually. The NIEHS colony will be retained only if disease-free animals are not available commercially and the special research requirements listed previously are sustained.

Approximately 30% of New Zealand White and Dutch Belted rabbits are infected with subclinical "snuffles" or Pasteurellosis. Recent initiation of strict screening practices at time of receipt seems to have been effective because fewer pneumonic rabbits are rejected prior to being issued for research. Persistent diarrhea, possibly due to Escherichia coli enterotoxemia, causes intermittent fatalities especially among NZW rabbits. Venereal spirochaetosis is not encountered in NZW rabbits; incidence in rabbits of the DB breed is very low.

Opossum deaths (approximately 10%) occur intermittently throughout the year, principally from bacterial endocarditis. The incidence of fatalities increases significantly during the breeding season (January - May) due to trauma and septicemia. Intestinal Salmonellosis (Poly B, Group F) predominates in approximately 70% of wild and captive-born animals but seems to be subclinical. Salmonella Poly D, Group R has been isolated from the tissues of septicemic animals and may be pathogenic along with various streptococci.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is a continuing effort to provide biomedical research with healthy laboratory animals acclimated to their environment. It is equally important to determine which diseases animals may have been exposed to and which lesions are associated with that disease. This data allows one to eliminate diseased animals from research and should help preclude the chance of misinterpreting data due to unknown intercurrent infections.

PROPOSED COURSE: Surveillance of laboratory animals will continue on a routine basis.

PUBLICATIONS

Gupta, B. N.: Meckel's diverticulum in a rat. Lab. Anim. Sci. (in press).

Serial No.: NIEHS-AST-007-4
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin"

PREVIOUS SERIAL NUMBER: NIEHS-AST-007-3

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.&A.H., Ph.D.; Joseph G. Zinkl, D.V.M., Ph.D.; J. G. Vos, Ph.D.; Julius E. Thigpen, Ph.D.

COOPERATING UNITS: Joseph Weissberg, M.D., Pathologic Physiology Branch

MAN YEARS

Total:	2.0
Professional:	1.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To identify the sequency of the toxicology processes through pathologic and clinical pathologic methods following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In addition, to identify abnormal processes associated with TCDD intoxication which might be investigated further in order to determine mechanisms of action of the toxic phenomena. Finally, to attempt to develop a biologic assay for determination of very low concentrations of TCDD in biologic material.

METHODS EMPLOYED: Rats, mice and guinea pigs were given single or multiple doses of TCDD orally in order to study the toxicologic, pathologic and clinical pathologic events of the intoxication. Standard techniques were used in order to evaluate the effects of TCDD.

A chick and/or quail tracheal cilia assay is being investigated as a possible method for the biological assay of TCDD.

MAJOR FINDINGS: A single oral dose of 100 µg/Kg of TCDD to CD rats caused death in 6 of 14 rats at a mean time of 18.3 days. Daily oral administration of 10 µg/Kg TCDD in 15/16 rats at a mean of 21.8 days after treatment has been commenced. Death occurred in 9/10 female Hartley strain guinea pigs after an average of 18.1 days after receiving 3 µg/Kg TCDD. Weekly oral doses at 1 µg/Kg caused death in 19 of 19 guinea pigs at a mean of 28 days after the initial treatment. A single oral dose of 1, 10 or 50 µg/Kg to CD-1 mice caused no deaths.

Rats given either 100 or 50 µg/Kg TCDD once or 10 or 1 µg/Kg weekly and C57Bl/6 mice given weekly doses of 25 µg/Kg of TCDD had weight losses or depressed weight gains. The only other notable clinical signs of toxicity were ruffled hair coat, hunched posture, inactivity and jaundice which appeared one or two days before death.

Clinical pathologic findings in rats receiving 1.0 and 10.0 µg/Kg of TCDD were increased SGPT and SGOT activities and elevated bilirubin cholesterol concentrations and hemoconcentration. Decreased platelet counts and serum glucose concentrations were also found.

In mice, leukopenia primarily due to lymphopenia was found one week after a single dose of 1.0, 10.0 or 50.0 µg/Kg TCDD. In guinea pigs treated weekly, lymphopenia was found in all groups. In addition, platelet counts were decreased in the 1.0 and 0.2 µg/Kg dose groups.

Investigation of the clotting mechanisms in rats given 10 µg/Kg TCDD revealed depression in clot retraction, increased platelet aggregation time, and extremely prolonged prothrombin consumption time.

Pathologically, the lymphoid organs had the most consistent alterations over the greatest dose range. Marked thymic atrophy was found and was characterized by decreased numbers of cortical thymocytes. Relative lymphoid depletion occurred in the spleen and lymph nodes. Degenerative and necrotic changes were found in the liver. Other changes found inconsistently in rats were hemorrhage in the gastrointestinal tract, liver, heart and brain; organized thrombi in different organs; and degenerative changes in renal connecting and collecting tubules and the thyroid. Icterus was also noted along with decreased abdominal and subcutaneous fat.

The most severely affected organ in the mice and guinea pigs was the thymus, which was markedly atrophied. Although liver lesions were found in these animals, they were not as severe as in rats.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: TCDD may be a contaminant in a variety of useful cyclic chlorinated compounds such as PCBs, pentachlorophenol, phenoxy acid herbicides and others. Abnormalities in immunocompetence, clotting mechanism or liver function occur at concentrations not detectable by current methods. These studies clearly show that control of the release of TCDD and other chlorodibenzo-p-dioxins in the environment is necessary.

PROPOSED COURSE: Work is in progress to determine the effect of TCDD on the immune mechanism (see NIEHS-AST-031) and the effect of TCDD on the clotting mechanism. Further work concerned with liver function will be initiated. In addition, similar investigations with other chlorodioxins are planned. Refinement and evaluation of the tracheal cilia assay method is to continue.

PUBLICATIONS

Harris, M., Moore, J., and Vos, J.: General biological effects of TCDD in laboratory animals. Environ. Health Perspectives, Experimental Issue No. 5,

Aug., 1973 (in press).

Zinkl, J., Moore, J., Vos, J., and Gupta, B.: Hematologic and clinical chemical effects of TCDD in laboratory animals. Environ. Health Perspectives, Experimental Issue No. 5, Aug., 1973 (in press).

Weissberg, J. and Zinkl, J.: Effects of TCDD on hematologic and blood coaggulation function in the rat. Environ. Health Perspective, Experimental Issue No. 5, Aug., 1973 (in press).

Gupta, B., Vos, J., Moore, J., Zinkl, J., and Bullock, B.: Pathologic effects of TCDD in laboratory animals. Environ. Health Perspectives, Experimental Issue No. 5, Aug., 1973 (in press).

Serial No.: NIEHS-AST-014-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Evaluation of a Captive-Born and Reared Colony of Opossums"

PREVIOUS SERIAL NUMBER: NIEHS-AST-014

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATOR: Julius E. Thigpen, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	0.8
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the physiologic, pathologic and reproductive parameters of a "closed" colony of captive-born opossums.

METHODS EMPLOYED: Opossums born during the 1972 breeding season (January - May) were caged individually in a "closed" facility. This colony was the second generation of captive-born young and consisted of 56 females and 46 males. All were free of helminths, as was the previous generation. Partitions separating 2 females and 1 male were removed January 5, 1972, and the animals permitted to breed at will. Vaginal smears were examined daily to record estrus, the duration of the estrus cycle and to detect sperm. Females were examined daily for young in the pouch, and records of births were maintained. All moribund and dead animals were necropsied and, if not autolyzed, major organs were cultured for pathogenic bacteria and mycoplasma.

MAJOR FINDINGS: The mortality rate, which was extremely low among adults prior to breeding, increased significantly during breeding. This is attributable to fighting with resulting septicemia. Bacteria cultured from vital organs have included salmonella, streptococci, pseudomonas and corynebacteria. The conception rate through 2 months of the breeding season is 23% as opposed to 46% among wild-trapped animals breeding in another facility. This difference may be due to the fact that a number of captive-born females were born in May, 1972, and as yet may not have reached sexual maturity. (By the end of the breeding season, the conception rate of captive-born versus wild animals may be comparable.) Fetal mortality within the first week of birth is approximately 50%, both in captive-born and wild animals. Considering the

relatively large number of young reaching the pouch at parturition, it is conceivable that a number may not properly attach to a nipple.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Maintenance of a closed breeding colony of captive-born opossums is feasible in providing sufficient animals for research from year to year. The added advantage of these animals is that they are helminth free. In addition, since they are born in captivity, they seem able to adapt to confinement better than a wild-captive animal. Further improvement and refinement of techniques associated with the use of opossums should be forthcoming.

PROPOSED COURSE: Continued evaluation of the biology of the captive opossum through FY 73. Development of the opossum model will be relegated a low priority due to more pressing NIEHS requirements.

Serial No.: NIEHS-AST-015-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Hematologic and Clinical Chemical Parameters in the Normal Virginia Opossum (Didelphis virginiana)"

PREVIOUS SERIAL NUMBER: NIEHS-AST-015

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATOR: Donald B. Feldman, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	0.35
Professional:	0.20
Other:	0.15

PROJECT DESCRIPTION

OBJECTIVES: To determine hematologic and clinical chemical parameters of normal opossums.

METHODS EMPLOYED: Blood samples were obtained from a closed colony of opossums. Routine clinical pathologic techniques were used for analysis.

In addition, 1-1/2 year-old opossums (3 males and 3 females) were given 2-^{14}C glycine in order to cohort label erythrocytes by incorporation of the ^{14}C into the heme portion of hemoglobin. Heme was isolated and the ^{14}C determined. The life-span of the erythrocytes can be determined from the time the radioactivity reaches a peak until it disappears.

MAJOR FINDINGS: Hematologic parameters, serum protein, protein fractions (albumin, α, β, δ, and δc globulin), BUN, glucose and cholesterol concentrations have been determined.

The erythrocyte life-span study is not yet complete.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because the use of opossums in research is continuing, it is imperative that investigators have methods of assuring themselves that their experimental animals are healthy, as well as methods of evaluating experimental conditions in these animals. Establishment of base-line data will help in these evaluations.

The study of the erythrocyte life-span will provide more knowledge about the physiology of the normal opossum. This project will add information and be useful in determining the reason many nucleated erythrocytes and reticulocytes are found in the blood of the opossum.

PROPOSED COURSE: The erythrocyte life-span is in the final process of completion after which the project will be terminated.

Serial No.: NIEHS-AST-018-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Effects of Polychlorinated Biphenyls on the Immunologic Response of Rabbits"

PREVIOUS SERIAL NUMBER: NIEHS-AST-018 and NIEHS-AST-019

PRINCIPAL INVESTIGATOR: Loren D. Koller, D.V.M., Ph.D.

OTHER INVESTIGATORS: Julius E. Thigpen, Ph.D. and Joseph G. Zinkl, D.V.M., Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.65
Professional:	0.15
Other:	0.50

PROJECT DESCRIPTION

OBJECTIVES: To ascertain the pathologic, immunologic and clinical pathologic effects of the polychlorinated biphenyls (PCBs) in the rabbit.

METHODS EMPLOYED: Male and female rabbits weighing about 2 Kg were given orally 300 mg of the PCB mixtures Aroclor 1221, 1242 or 1254 weekly for 13 weeks. During this period, biweekly blood samples were taken for clinical pathologic analysis. Commencing with the 10th week, rabbits were inoculated three times with formalinized Pseudorabies virus (PRV) at one week intervals. Serum neutralization tests using RK-13 monolayers and a standard dose of Pseudorabies virus was used to determine endpoint titers of antiserum to PRV utilizing serum collected before and 7 and 24 days after the initial antigen injection. The rabbits were killed at 15 weeks and tissues were taken for histopathology and electron microscopy.

MAJOR FINDINGS: Rabbits given Aroclors 1221, 1242 and 1254 had lower serum neutralizing antibody titers to PRV than rabbits not exposed to the PCBs. The titers were approximately one-half those of the controls, and rabbits given Aroclor 1242 had the lowest titers of the three PCBs used.

Clinical pathologic findings included elevated SGOT and SGPT activities as early as 2 weeks in the 1254 and 1242 treated rabbits. Serum cholesterol concentrations were moderately elevated in the 1254 males. The livers of the 1254 and 1242 rabbits were enlarged. The earliest change was megalohepatocytosis which was followed by subcapsular and midzonal necrosis. In the 1254

rabbits, the midzonal necrosis was diffuse and frequently included the central part of the lobule. The rough endoplasmic reticulum in the livers of the 1254 rabbits was destroyed. Uterine atrophy occurred in the 1254 treated rabbits, also.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1254 and 1242 were hepatotoxic, 1254 also caused uter ne atrophy whereas 1221 was not toxic at the dose given, indicating a difference in the toxicity of the Aroclors. However, immunosuppressive effects were found for all the PCBs. These results suggest that toxicity of PCBs and effects on the immune mechanism are not related.

The difference in toxicity might be useful in replacing some PCBs with less toxic PCBs for industrial use.

PROPOSED COURSE: Project complete. Manuscript concerned with the immunologic effects is in preparation.

PUBLICATIONS

Koller, L. D. and Zinkl, J. G.: Pathology of polychlorinated biphenyls in rabbits. Am. J. Path. 70: 1973.

Serial No.: NIEHS-AST-020-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Effect of Acute Starvation after Long-Term, Low-Level Feeding of a Polychlorinated Biphenyl Mixture (Aroclor 1254) in Rats"

PREVIOUS SERIAL NUMBER: NIEHS-AST-020 (This is a revision of this project in that it was switched from opossums to rats)

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.55
Professional:	0.25
Other:	0.30

PROJECT DESCRIPTION

OBJECTIVES: To study the effect of starvation on mobilization of PCBs stored in body fat.

METHODS EMPLOYED: Female rats weighing approximately 175 grams were fed Aroclor 1254 in the diet at 10, 30 and 100 ppm. After 2, 5, 8, 12, 16 and 20 weeks five animals in each group were bled and killed for clinical pathologic and histopathologic determinations. After 20 weeks, one-half the animals were starved (no food) and one-half were given rat laboratory chow free of PCBs. During this period at 3, 6, 10 and 13 days, rats were again bled for clinical pathologic and histopathologic analysis.

MAJOR FINDINGS: Rats at the highest dose did not gain weight as rapidly as the controls. Some rats (10%) at both 30 and 100 ppm developed skin lesions at about 9 weeks on the ears, nose, tail, and feet. After 8 weeks, rats on 30 and 100 ppm diet had enlarged liver which fluoresced under U.V. light, indicating that porphyria had developed. No clinical pathologic changes were found.

After starvation, the rats had increased SGPT and SGOT activities. Further histopathologic and clinical pathologic analysis has not been completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Caloric deprivation occurs in man at times. In addition, anorexia commonly occurs in people with severe illness. If these people have PCBs in their fat tissue, mobilization could occur leading to hepatotoxicity and acquired porphyria.

PROPOSED COURSE: Project will be continued until all analyses are complete.

Serial No.: NIEHS-AST-022-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Evaluation of Teratogenic Effects of Hycanthone Methanesulfonate and Selected Analogs in the Mouse"

PREVIOUS SERIAL NUMBER: NIEHS-AST-022

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.9
Professional:	0.1
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: To determine the effects hycanthone methanesulfonate and other thioxanthone derivatives have on the developing fetus.

METHODS EMPLOYED: Using standard teratological techniques, attempt to determine the doses at which no fetal effect to fetal death occurs. By varying dose within these limits, as well as the gestational period during which dose is administered, determine by visceral and skeletal examination of fetuses what effect the compound has on fetal development.

MAJOR FINDINGS: Hycanthone methanesulfonate is embryotoxic and teratogenic to the developing rat fetus. Similar findings have been previously reported in mice.

Limited investigation in the mouse with the chloroindazole analog failed to demonstrate any teratogenic effect.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hycanthone methanesulfonate, a drug currently being used in the treatment of schistosomiasis is teratogenic in mice and rats. Preliminary findings indicate that the chloroindazole analog of hycanthone, which also has schistosomicidal properties, is not teratogenic.

PROPOSED COURSE: Other thioxanthone derivatives will be tested, primarily through contract activities, for teratogenic potential. The structure activity

relationships found will be correlated with the known mutagenic properties for the same compounds.

PUBLICATIONS

Moore, John A.: Teratogenicity of hycanthone in mice. Nature 239: 107-109, No. 5367, September 8, 1972.

Serial No.: NIEHS-AST-023-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin"

PREVIOUS SERIAL NUMBER: NIEHS-AST-023

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.&A.H., Ph.D. and Joseph Zinkl, D.V.M., Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Continuous treatment of pregnant mice with TCDD results in production of cleft palate and hydronephrosis in their offspring. This study is to specifically focus on the following:

1. Characterize the stages of development during which the rodent fetus is susceptible to TCDD.

2. Characterize the pathogenesis of renal malformations due to TCDD treatment.

METHODS EMPLOYED: Using timed pregnant C57Bl/6 mice, varying doses of TCDD are administered per os at specific periods of gestation. Treated females are killed at various intervals; the fetuses are harvested and subsequently examined. In postnatal studies, reciprocal cross foster manipulations are used to determine renal effect due to *in utero* exposure, postnatal exposure or combined exposure.

MAJOR FINDINGS: Maternal exposure of C57Bl/6 mice to TCDD caused variations in fetal kidney maturation and development when compared to control. This response was dose related as scored by the number of fetuses affected and whether kidney involvement was unilateral or bilateral. In addition, the thymuses from treated litters were reduced in size although no effect on fetal weight was observed. To distinguish between effects due to a reversible maturational delay and permanent development effects, postnatal studies were

initiated. Hydronephrotic kidneys developed postnatally in mice whose mother was exposed to TCDD during pregnancy or at the time of birth. The highest incidence occurred in those mouse pups who nursed a mother treated at birth or whose mother was treated during pregnancy and subsequently nursed her young. Mean body weight of litters nursing mothers treated with 10 µg/Kg TCDD at birth was less than that of controls. Absolute and relative thymus and spleen weights were also reduced. No effect on body weights or organ weights was seen in pups whose mother received 3 µg/Kg TCDD at birth. Kidney effects were seen at both dose levels.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The etiology of the vast majority of renal malformation remains obscure. Determining the mechanism by which TCDD produces this effect may help define factors which lead to kidney malformation. The exquisite susceptibility of the developing C57Bl/6 mouse kidney to TCDD affords a reproducible system to study this disease. Significance of depressed thymus weight as it effects cellular immunity will be evaluated.

PROPOSED COURSE: Project to continue.

PUBLICATIONS

Moore, John A., Gupta, B. N., Zinkl, J. G., and Vos, J. G.: Postnatal effects of maternal exposure to TCDD. Environmental Health Perspectives, Experimental Issue No. 5, Aug., 1973 (in press).

Serial No.: NIEHS-AST-025-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Comparative Effects of Selected Detergent Formulations on the Rabbit Eye"

PREVIOUS SERIAL NUMBER: NIEHS-AST-025

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATOR: John A. Moore, D.V.M.

COOPERATING UNITS: Industrial Biotest Laboratories, Inc.
Northbrook, Illinois

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To determine the extent of eye damage following installation of phosphate, carbonate or metasilicate containing detergents into the rabbit eye.

METHODS EMPLOYED: The FDA Standard Eye Test was modified by extending the observation period to 14 days. The detergents, in dry form (0.1 ml), were inserted between the lower lid and cornea; the eye was gently held shut for one second and not washed. Control and experimental eyes were graded for extent of damage (Draize) and photographed. In selected instances, both eyes (experiment and opposite control) were removed and examined for histopathologic changes. Nine commercial products were tested.

MAJOR FINDINGS: There appeared to be, with minor variations, a positive correlation between the type and extent of eye damage observed with the composition of the product's active ingredient. All products produced significant eye irritation. Major significance, however, was placed on the potential for producing residual corneal damage because this type of lesion would impair vision.

The phosphate containing detergents caused a transient superficial corneal opacity which existed a maximum of 42 hours. All eyes were found to be essentially normal by the end of the 14-day observation period.

Eyes treated with a carbonate containing detergent typically developed a marked corneal opacity which persisted throughout the observation period. In some cases, the integrity of the corneal surface was compromised with the production of a desmetocele.

Metasilicate containing detergents are characterized by their marked caustic properties which produce irreversible eye damage. At the end of the 14-day observation period, either total eye destruction or complete corneal opacity was observed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study demonstrates that certain household detergents present a real hazard in their potential ability to produce permanent eye damage.

PROPOSED COURSE: Project completed.

PUBLICATIONS

Feldman, D. B., Rall, D. P., and Moore, J. A.: Dry detergent and soap effects on the rabbit eye. Journal of the American Medical Association 221: 9, 1055, 1972.

Serial No.: NIEHS-AST-026-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Transmission and Pathogenesis of Encephalitozoan cuniculi in the Rabbit"

PREVIOUS SERIAL NUMBER: NIEHS-AST-026, NIEHS-AST-027, and NIEHS-AST-028

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: Julius E. Thigpen, Ph.D. and John A. Moore, D.V.M.

COOPERATING UNITS: Dr. John Shadduck, Ohio State University

MAN YEARS

Total:	0.8
Professional:	0.2
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: Laboratory propagation of Encephalitozoan cuniculi for the purpose of detecting the parasite, and for determining its transmission and pathogenesis.

METHODS EMPLOYED: Viable organisms were inoculated onto confluent monolayers of rabbit choroid plexus cells at a concentration of 10 organisms per cell. Infected cultures were harvested in 7 to 21 days and frozen at -70°C to release Nosema organisms. Supernatants from thawed cultures were pooled, the organisms purified by centrifugation and their numbers counted in a chamber.

Rabbits negative to Nosema (skin test) were to be hyperimmunized by various routes with Nosema antigen harvested from infected rabbit choroid plexus cells grown in vitro. Both hyperimmunized and incoming rabbits of unknown infection were to be skin tested and bled for the purpose of comparing the skin test with the Indirect Fluorescent Antibody (IFA) test. Groups of adult female NZW rabbits, negative to Nosema (skin test), were to be experimentally inoculated with Nosema by various routes. Unborn and new born young were to be examined for Nosema lesions employing histological and fluorescent antibody techniques. Other young were to nurse respective does or to be cross-fostered to preclude lactation transmission. Reciprocal cross-fostering would determine infectivity during the nursing period. All surviving offspring were to be skin tested and groups were to be necropsied at intervals for a period of 6 months.

MAJOR FINDINGS: Although Nosema has been successfully propagated in massive numbers, preliminary $TCID_{50}$ endpoints indicate less than 0.001% infectivity. The reasons for low infectivity are unknown and will require further investigation before successful testing, pathogenesis and transmission studies can be completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Encephalitozoonoses has been detected in our research rabbits, producing renal and cerebral lesions which, though clinically silent, may be an experimental variable. Elimination of infected animals is desirable, thus sensitive screening tests must be evaluated and perfected. As rabbit offspring and ova are used prominently in research at the Institute, it is necessary to determine the pathogenesis and natural transmission of the organism.

PROPOSED COURSE: To continue propagation, infectivity and transmission studies as planned and to evaluate the data for publication.

Serial No.: NIEHS-AST-030-2
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of Environmental Chemicals on Experimental Viral Infections in Mice"

PREVIOUS SERIAL NUMBER: NIEHS-AST-030

PRINCIPAL INVESTIGATOR: Joseph H. Gainer, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1
Professional:	1
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: To test for interactions in mice between the administration of an environmental chemical, principally the salt of a heavy metal, and a virus infection. Where interactions were shown, studies were performed on interferon (IF) synthesis and action, since IF is one of the first lines of defense against a virus infection.

METHODS EMPLOYED: Maximum concentrations of metallic salts were used which the mice could tolerate without resulting in death or appreciable reduction in rate of growth or in liquid consumption. They were administered continuously in the drinking water to CD-1 male mice beginning at 4 weeks of age. At 6 weeks of age, the mice were inoculated intraperitoneally with an LD_{10} to 20 of the encephalomyocarditis virus (EMCV) and patterns of death were recorded. Minimum concentrations of $HgCl_2$ were determined that would still enhance EMCV mortality.

Metals that had previously been shown to increase EMCV mortality were tested in several ways for their effects on IF function. They were tested as follows: a) for their influence on IF action in the intact mouse; b) for their effect on Poly I/Poly C's protection to EMCV mortality; and c) for their effect on the protection of Newcastle disease virus (NDV) against EMCV mortality. In the case of (a), mouse IF was prepared in the intact mouse by administering 10 mg/Kg Poly I/Poly C and bleeding and killing the mouse six hours later for plasma, splenic and pulmonary IF. Other mice were treated with the metals and with the mouse IF followed by challenge with the EMCV. In (b), Poly

I/Poly C, 0.1 mg/Kg [a dose which reduced an LD_{95} of EMCV to an LD_5] was administered intraperitoneally (IP) to controls and the metal-treated mice followed two hours later with an LD_{95} of EMCV. In (c), control and metal-treated mice were given 1 percent body weight IP of NDV [LaSota strain, 1 - 1000 dose vial of commercial vaccine, American Scientific Laboratories, Madison, Wisconsin, dissolved in 100 ml sterile saline]. Twenty-four hours later, the mice were inoculated IP with an LD_{95} of EMCV. Rates of mortality were computed and effects of the metals in IF formation or action were determined. In (b) and (c), it is assumed, although not clearly proved, that the protection against EMCV mortality afforded by Poly I/Poly C and NDV is through the synthesis and action of IF.

MAJOR FINDINGS: $HgCl_2$ at 1 ppm in the drinking water significantly enhanced EMCV mortality, but 0.4 ppm and below did not have any effect.

None of the metals tested—As, Cd, Hg, Ni, or Pb—depressed the action of IF in the intact mouse. However, As, Co, and Pb depressed Poly I/Poly C's protection to EMCV mortality whereas Cd, Hg, and Ni did not do so. Ni and Pb inhibited the protection by NDV against EMCV mortality and Cd and Hg were suspect in this regard; As and Co were without any effect on NDV's protection to EMCV mortality.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: That 1 ppm $HgCl_2$ was stimulatory to viral infection suggests that studies on mercury intensification of human viral infections should be considered. This 1 ppm level of mercury is commonly exceeded in certain seafoods.

The interactions of the metallic salts with two different interferon inducers pinpoint with some precision certain cells of the body which may or may not be influenced by the metal. E. De Maeyer Guignard (Science, 1972, 177: 797) illustrated three different cell types in the intact mouse that could produce IF depending upon the inducer. NDV stimulated IF production in the radiosensitive lymphocyte, Poly I/Poly C induced IF production in a radioresistant small macrophage, and the EMCV induced IF production in another kind of radioresistant small macrophage. From data obtained here, we may conclude that arsenic, cobalt, and lead poison Poly I/Poly C's induction of IF in small macrophages whereas cadmium, mercury, and nickel have no effect. In addition, lead and nickel (and possibly cadmium and mercury) poison NDV's induction of IF in lymphocytes, but arsenic and cobalt had no effect. Quantitatively, lead influenced Poly I/Poly C's induction of IF more so than it did NDV's induction of IF. Another possible conclusion that may be arrived at, subject to experimental testing, is that lead and nickel, both of which inhibited NDV's induction of IF, might be expected to synergize with gamma rays (or with radiomimetic drugs) in that they produced a functional inhibition of IF synthesis in radiosensitive lymphocytes.

PROPOSED COURSE: To continue studies of this nature as time permits.

PUBLICATIONS

Gainer, J. H.: Increased mortality in encephalomyocarditis virus-infected mice consuming cobalt sulfate: Tissue concentrations of cobalt. Amer. J.

Vet. Res. 33: 2067-2073, 1972.

Gainer, J. H., Pry, T. W.: Effects of arsenicals on viral infections in mice. Amer. J. Vet. Res. 33: 2299-2307, 1972.

Gainer, J. H.: Effects of arsenicals on interferon formation and action. Amer. J. Vet. Res. 33: 2579-2586, 1972.

Serial No.: NIEHS-AST-031
1. Animal Science and Technology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of Environmental Chemicals on Immunocompetence"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. G. Vos, Ph.D.

OTHER INVESTIGATOR: John A. Moore, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	2.5
Professional:	1.3
Other:	1.2

PROJECT DESCRIPTION

OBJECTIVES: Determination and evaluation of inhibitory effects of environmental chemicals on the immune system.

METHODS EMPLOYED: Humoral immunity is assessed by the determination of antibody titers in vaccinated animals using a modified single radial immunodiffusion (Mancini) technique and a passive haemagglutination method. Cell-mediated immunity is tested in vivo by a skin test (delayed type hypersensitivity to tuberculin), the graft versus host activity of donor lymphoid cells injected into F_1 hybrid recipients (lymph node weight assay) and in vitro by the mitotic response of lymphocyte cultures to PHA as evaluated by uptake of ^{3}H-thymidine.

MAJOR FINDINGS: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) depressed the cell-mediated immunity (skin test) in guinea pigs at a very low, sublethal dose level (8 weekly doses of 0.04 µg TCDD/Kg body weight). No inhibitory effect was found in rats using the skin test, despite a marked thymic atrophy. In mice, a dose-related reduction was found in the graft versus host activity of spleen lymphoid cells from donor mice treated with sublethal levels of TCDD.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is important to establish possible effects of environmental contaminants on the immune system, and to determine the mode of action, in order to evaluate the hazards of immune suppression for man and animal.

PROPOSED COURSE: Work with TCDD will be continued. Whether TCDD-induced

suppression of cell-mediated immunity in guinea pigs and mice (the most significant pathologic lesions are found in the lymphoid organs) plays a major role in the death of these animals will be researched. Thymus grafts in TCDD treated mice will be used to determine whether there is a protective effect on mortality. Pregnant mice will be dosed with TCDD to determine the effects on the immune system of their offspring.

An indirect fluorescent antibody technique will be used to determine the percentage of T (thymus dependent) lymphocytes in peripheral blood, in order to evaluate the TCDD-induced lymphopenia.

Moreover, some other compounds (chlorinated dibenzofuran and methyl mercury) will be studied.

PUBLICATIONS

Vos, J. G., Moore, J. A., and Zinkl, J. G.: Effect of TCDD on the immune system of laboratory animals. Environmental Health Perspectives, Experimental Issue No. 5, August 1973 (in press).

Serial No.: NIEHS-AST-032
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Development of Analytical Methodology"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-006

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.; E. O. Oswald, Ph.D.; R. Thomas; J. Fawkes; A. Latimer

COOPERATING UNITS: None

MAN YEARS

Total:	0.50
Professional:	0.50
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: To develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include (1) those for which no analytical methodology, insufficiently specific or sensitive methodology, or no methodology we are equipped to utilize are presently available; (2) those which are metabolites of compounds for which adequate analytical methodology is available, and (3) those for which an adequate analytical procedure is available but which occur in a milieu providing interference with the standard procedure.

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorimetry, spectrophotometry (IR, UR, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS: (1) Polychlorinated Biphenyls (PCBs): These compounds, providing 209 closely related species and isomers, provide an extremely sensitive probe for charge-transfer and dipole-induced dipole interactions in GLC. Work has continued on the development of a retention-index-based technique for evaluating these interactions, but additional work needs to be done. A combined GLC-IR "fingerprinting" technique was used successfully to measure levels of PCBs in microscope immersion oils. (2) Methyl Mercury: Gas

chromatographic methods for the determination of methyl mercury in tissues were evaluated and found to have in common one thing - poor recovery. A modified method using double internal standards was developed to overcome this problem. (3) Complete methodology for the study of phthalates appears to be at hand. (4) Piperonyl butoxide aerosol sampling methods were evaluated by GLC analysis of recovered synergist.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Section as well as be of utility for other studies within and without the Institute.

PROPOSED COURSE: (1) There is presently no adequate technique for the determination of "total PCB" in residue or extracts when the isomeric composition does not happen to match one of the commercial PCB mixtures. We will attempt to develop such a technique. (2) We will develop analytical methodology to meet the other objectives.

PUBLICATIONS

Fishbein, L.: Chromatographic and biological aspects of polychlorinated biphenyls. J. Chromatog. 68: 345-426, 1972.

Fishbein, L., and Albro, P. W.: Chromatographic and biological aspects of phthalate esters. J. Chromatogr. 70: 365-412, 1972.

Serial No.: NIEHS-AST-033
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Identification and Characterization of Potentially Hazardous Environmental Agents by Mass Spectrometry"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-039

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: P. W. Albro, Ph.D. and J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Our present computerized Mass Spectrometry facilities, including the Finnigan 1015 Electron Impact system and the Finnigan 1015 Chemical Ionization unit afford the capability of collecting and correlating a tremendous amount of spectral information. Because chemical ionization mass spectrometry is most useful in direct complement with electron impact data, all of the compounds are analyzed and correlated on both systems. This catalogued information will serve as an identification library of various organic environmental agents.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), I.R., N.M.R. and mass spectrometry, computer analyses, organic syntheses.

MAJOR FINDINGS: Probably the most versatile analytical technique available is gas-liquid chromatography; however, by this method alone one cannot conclusively verify the chemical structure of a compound. By use of a combined gas-liquid chromatographic system which is directly interfaced to a mass spectrometer, one is able to thoroughly examine the chemical structure of microgram and even low nanogram quantities of environmental agents and their metabolites. In numerous cases because of the polyfunctionality and chemical nature of a compound, even by use of very low energy electron bombardment potentials of 20 volts or less, the mass spectrum may not contain all of the required mass fragments and the molecular ion necessary for positive identification. By use of chemical ionization mass spectrometry combined with gas-liquid chromatography, one is able to obtain the desirable fragment in the

area of the molecular ion and other pertinent mass fragments which unequivocally identify the particular compound even in low nanogram quantities. By the complementary characterization of crudely purified environmental agents from biological samples by combined gas liquid chromatography with chemical ionization and electron impact mass spectrometry, one is able to identify the chemical structure of many polyfunctional metabolites and reaction products of potentially hazardous environmental agents.

We have applied this complementary use of chemical ionization and electron impact mass spectrometry interfaced with gas liquid chromatography to: (1) chlorinated hydrocarbons including DDT derivatives; polycyclodienes including dieldrin, aldrin and metabolites; and polychlorinated biphenyls (PCB); (2) branched chain hydrocarbons and metabolites including tertiary alcohols; (3) plasticizers including the rat urinary metabolites of di-(2-ethylhexyl)phthalate; (4) prostaglandins; (5) amines and amino acid derivatives; and (6) allyl and propenyl benzenes including their urinary metabolites - tertiary aminopropiophenones.

Numerous of the above identification problems associated with metabolic investigations would not have been undertaken without this complementary use chemical ionization and electron impact mass spectrometry.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many deficiencies in our knowledge concerning the metabolic fate of phthalates, polychlorinated biphenyls, pesticides, amines, organometallic compounds and numerous other hazardous environmental agents have existed for a number of years. By the use of this powerful analytical tool, chemical ionization and electron impact mass spectrometry, some of these scientific findings have greatly decreased deficiencies of knowledge in these areas.

PROPOSED COURSE: (1) To continue to characterize numerous environmental agents and their metabolites by chemical ionization and electron impact mass spectrometry. (2) To update the mass spectrometer and data retrieval systems. (3) To develop a multiple ion detection capability for quantitative analyses of picogram quantities of specific environmental agents.

PUBLICATIONS

Oswald, E. O., Fishbein, L., Corbett, B. J., and Walker, M. P.: Metabolism of naturally occurring propenyl benzene derivatives. II. Separation and identification of tertiary aminopropiophenones by combined gas-liquid chromatography and chemical ionization mass spectrometry. J. Chromatogr. 73: 43-59, 1972.

Oswald, E. O., Fishbein, L., Corbett, B. J., and Walker, M. P.: Chemical lability of the tertiary aminopropiophenones of eugenol as characterized by combined gas-liquid chromatography and chemical ionization mass spectrometry. J. Chromatogr., 73: 59-72, 1972.

Albro, P. W., Thomas, R., and Fishbein, L.: Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr., 1973 (in press).

McKinney, J. D., and Oswald, E. O.: Characterization of chlorinated hydrocarbon pesticide metabolites of the DDT and polycyclodiene types by electron impact and chemical ionization mass spectrometry. (For presentation at the 165th ACS National Meeting, Dallas, Texas, April, 1973).

Serial No.: NIEHS-AST-034
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Assessment and Development of Methods of Metal Analysis"

PREVIOUS SERIAL NUMBER:

PRINCIPAL INVESTIGATOR: M. D. Folsom, Ph.D.

OTHER INVESTIGATOR: J. Fawkes

COOPERATING UNITS: None

MAN YEARS

Total:	.75
Professional:	.25
Other:	.50

PROJECT DESCRIPTION

OBJECTIVES: To investigate and increase the accuracy, sensitivity and range of usefulness of standard methods of metal analyses of projected usefulness for biological studies.

METHODS EMPLOYED: Gas chromatography, atomic absorption, thin-layer chromatography.

MAJOR FINDINGS: Gas chromatography was useful for relatively accurate determination of methyl mercury. Benzene extracts (1:1 ratio to acidified homogenate) and an internal standard were most satisfactory. At levels greater than 10 ppm gas chromatography gave high values. This, as well as the 10% standard error, made this method unsatisfactory for use in comparison of total with inorganic and organic mercury. Extraction of the mercury from acidified homogenate with dithizone as methyl mercury dithizonate and mercury dithizonate, with thin-layer chromatography of the dithizone extract, elution of the fractions, and subsequent analysis by atomic absorption gave more precise results which could be compared.

Sensitivity and precision of atomic absorption analyses for Hg was found to be much increased by the amalgamation of the Hg on a silver gauze trap. After interfering substances were passed through the system, the Hg was released by heating.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Past problems with inaccuracy of methods of analysis of metals in biological

samples, particularly with small amounts and background levels, have made data hard to interpret and results of experiments difficult to compare. Reliable analytical methods are necessary for biological effects to be completely understood.

PROPOSED COURSE: Similar analytical work on cadmium is underway.

Serial No.: NIEHS-AST-035
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Chemistry of Biphenyls, Chemical Prototypes Including the Synthesis of Labeled and Unlabeled PCBs and Their Probable Metabolites"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-043

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D., L. Levy, Ph.D., M. Walker, and C. Ford

COOPERATING UNITS: None

MAN YEARS

Total:	2.0
Professional:	1.2
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: To develop model chemical hydroxylating systems for PCBs in order to reveal their peculiar electronic nature and provide small quantities of synthetical metabolites for analytical comparison with authentic metabolites. To note differential behavior in the model chemical hydroxylation of biphenyls and the usual benzenoid systems. To determine if stereochemical factors associated with the more highly substituted PCBs could effect differences in metabolism and toxicity. To synthesize where possible desired ^{14}C-labeled PCBs and their metabolites.

METHODS EMPLOYED: Organic syntheses, chromatography (TLC, column and GLC), spectroscopy (IR, NMR and MS), radioactive monitoring and quantitation.

MAJOR FINDINGS: The utility of chemical models for study of oxidative enzymes has provided considerable insight into the mechanism of oxidation of the aromatic ring with subsequent formation of phenols. Preliminary experiments in our laboratory suggest that such model reactions are applicable to the PCBs including photooxygenation to simulate weathering. Regiospecific hydroxylation of biphenyl and two monochlors has been accomplished. Reasonable quantities of the products have been isolated for use as analytical standards. The same experiments performed on ^{14}C-labeled materials have afforded labeled synthetical metabolites. Although there is no evidence to support it at this time, it seems likely that cationoid and arene oxide-oxepin intermediates are formed as

in the usual benzenoid systems such as naphthalene. The ^{14}C-labeled monochlors have been prepared to facilitate diverse metabolism studies. The synthetic methodology previously developed for unlabeled materials has been modified accordingly for labeling. In addition, specific synthetic methodology for the more highly substituted PCBs is being developed including the unique synthesis of potentially optically active members of the PCBs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The commercial PCBs are known to be complex mixtures and are recognized to be of major environmental importance due to their ubiquitous ecological distribution and concentration in the food chain. Availability of purified labeled and unlabeled members of the PCBs and their probable metabolites would permit more definitive biological and toxicological studies and, hence, allow a more accurate assessment of their potential hazard. Model chemical hydroxylation studies would also illuminate peculiar stereoelectronic properties, which affect their metabolism, of the individual PCBs.

PROPOSED COURSE: To synthesize additional labeled and unlabeled PCBs. To subject all available purified PCBs (labeled and unlabeled) to model chemical hydroxylating conditions. To determine relative oxidative propensities of these individual PCBs. To determine the relative degree of the arylating ability of the suspected arene oxide-oxepin metabolic intermediates of the individual PCBs where possible.

PUBLICATION

Levy, L. A.: Synthesis of unsymmetrical polychlorinated biphenyls. Synthesis, 1973 (in press).

Serial No.: NIEHS-AST-036
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Polychlorinated Biphenyls"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-045

PRINCIPLA INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D.; L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the mammalian and microbial metabolism of labeled and unlabeled chlorobiphenyls.

METHODS EMPLOYED: A variety of chromatographic and mass spectroscopic techniques.

MAJOR FINDINGS: In the temporary absence of suitable ^{14}C-labeled polychlorinated biphenyls, we have been attempting to refine analytical methodology for PCBs. Thus far infrared spectroscopy has proven very useful for measurement of PCB mixtures, but TLC has been of little or no use. Other findings will be reported by the other investigators.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Unequivocal identification of the metabolites and their tissue distribution, as well as knowledge of the rates of absorption and elimination of the isomeric polychlorinated biphenyls, is essential for the assessment of the potential hazard of these ubiquitous environmental agents.

PROPOSED COURSE: Identification and characterization of the metabolites of a selected number of labeled and unlabeled isomeric chlorobiphenyls derived both from mammals and bacteria. Development of an analytical method capable of measuring "total PCB" when the isomeric composition is unknown No such method exists at the present time.

PUBLICATIONS

Albro, P. W., and Fishbein, L.: Intestinal absorption of polychlorinated biphenyls in rats. Bull. Environ. Contam. & Toxicol. 8: 26-31, 1972.

Albro, P. W., and Fishbein, L.: Quantitative and qualitative analysis of polychlorinated biphenyls by gas-liquid chromatography. I. One to three chlorine atoms. J. Chromatogr. 69: 273-283, 1972.

Serial No.: NIEHS-AST-037
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Nuclear Magnetic Resonance Studies of Substituted Biphenyls"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-036

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATOR: Marshall Anderson, Ph.D.

COOPERATING UNITS: Biometry Branch

MAN YEARS

Total:	0.7
Professional:	0.6
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To study the detailed molecular and electronic structure of several substituted biphenyls and their possible interactions with complexing agents which may be of biological importance.

METHODS EMPLOYED: Variable temperature NMR and supportive computer methodology.

MAJOR FINDINGS: The relationships between NMR parameters and the calculated (CNDO/2) electron densities in several symmetric chlorinated biphenyls have been found. Preferred conformations for these biphenyls have been predicted from the nmr data.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies should help to illuminate the types of interactions in which the substituted biphenyls can participate. The polychlorinated biphenyls, in particular, are of wide current interest as environmental hazards. This work should increase our understanding of how they may be biologically active.

PROPOSED COURSE: This work will continue, and will be extended to include biphenyls with a greater degree of substitution and asymmetry.

PUBLICATIONS

Wilson, N. K., Anderson, M., and Stothers, J. B.: Magnetic resonance spectra and approximate molecular orbital calculations for symmetric chlorinated biphenyls. J. Amer. Chem. Soc. (In preparation)

Wilson, N. K., and Anderson, M.: Carbon-13 and proton magnetic resonance studies of chlorinated biphenyls. Proceedings of the Symposium on Mass Spectrometry and NMR in Pesticide Chemistry, American Chemical Society, Dallas, April, 1973. (In press)

Serial No.: NIEHS-AST-038
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Chemical Synthesis of Select Metabolites and Possible Metabolic Intermediates of Dieldrin and Related Polycyclodiene Pesticides"

PREVIOUS SERIAL NUMBER:

PRINCIPAL INVESTIGATOR: S. M. dePaul Palaszek, Ph.D.

OTHER INVESTIGATOR: J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To utilize dieldrin and related polycyclodiene pesticides as chemical models to elucidate the mechanisms of certain biological oxidations. To synthesize F-1 and U-1 by methods which might mimic enzymatic behavior. To synthesize possible intermediates in dieldrin metabolism for toxicity study and contribution to the knowledge of enzymatic oxidative mechanisms.

METHODS EMPLOYED: A variety of synthetic procedures, and instrumental techniques including glc, TLC and column chromatographic separations and identification and ir, nmr, UV, and ms characterization and identification.

MAJOR FINDINGS: (a) Direct oxidation of dieldrin or aldrin with chromyl acetate, hydride abstraction with DDQ or trityl cation, and one-electron oxidation with perbenzoate-cuprous iodide or pyridine-1-oxide were unsuccessful in producing either the major urinary (U-1) or fecal metabolite (F-1) of dieldrin or their precursors. The NIH shift phenomenon associated with enzymatic oxidations had been reported for several of these reagents. Although the reactions attempted did not produce the desired results, they have demonstrated the stability of the methylene group, the preferential reaction at the epoxide ring site, and the dissimilarity between epoxide ring opening under intimate ion pair conditions (H_2SO_4-Ac_2O) and Lewis acid catalyzed ring opening (trityl cation-HOAc). Cis-Dihydroxydihydroaldrin (CAD), the preferred configuration under intimate ion pair conditions, has been shown in other work to be the major enzymatic hydration product of dieldrin. The Chau and Cochrane

synthesis of 4,5-diacetoxyphotodieldrin reproduced in this study has been the only reported chemical reaction to produce the bridged structure that exists in U-1 and in photodieldrin. The stereochemistry of the acetoxyl groups, heretofore unknown, is under study. The stereochemistry will provide evidence to support one or another structure for the intermediate that leads to the bridged product. A reagent that models certain oxygenase enzyme reactions, trifluoroacetic acid-hydrogen peroxide-boron trifluoride, has been successfully used to open the stable dichloroethylene bond of dieldrin. The exact structure of the hydroxy ester isolated has not been determined. However, the possibility of effecting collapse of the ester to U-1 exists because there are a limited number of structures possible. The enzymatic epimerization of CAD to TAD in vitro has been established in other work. The intermediacy of an α-hydroxy ketone or the isomeric ene-diol in the epimerization is mechanistically reasonable. Attempts to produce the ene-diol or hydroxy ketone from TAD or CAD with chromium reagents produced substantial loss of material. Successful DMSO oxidations of steroids, alkaloids, and carbohydrates have been reported. Dimethylsulfoxide (DMSO)-acetic anhydride oxidation of CAD produced two major products. Evidence (chemical conversions, ir, ms, glc, tlc and nmr) suggests exo-hydroxy ketone and ene-diol structures for the products. The ene-diol was also produced from the DMSO-BF_3(g) oxidation of dieldrin. No dioxopolycyclodiene was isolated and attempts to form it from the ene-diol or hydroxy ketone were unsuccessful. The ene-diol and hydroxy ketone are structurally amenable to chelation, a possible model for enzyme-substrate interaction. Conditions for chelation are under study.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polycyclodiene pesticides are persistent insecticides in our environment. The rigid polycyclodiene system provides an attractive chemical model for studying enzyme reactions. The enzymatic stereospecific epoxide ring opening and stereoselective epimerization are explicable on the molecular level. The major urinary metabolite (U-1) and major fecal metabolite (F-1) of dieldrin must represent some specific enzyme involvement because a variety of oxidizing conditions have failed to produce these metabolites or their possible precursors. The intermediates in these biological transformations may mediate toxic effects greater than the pesticide or its metabolites. Dieldrin and its metabolites are stereochemically similar to certain male sex hormones, and it has been demonstrated that dieldrin metabolism is affected by the presence of testosterone. An understanding of enzyme mechanisms on the molecular level as well as the possibility of enlightened design of therapeutic agents are to be gained by a study of chemical systems that mimic enzyme behavior.

PROPOSED COURSE: To pursue the synthesis and characterization of proposed intermediates in the metabolism of dieldrin. To investigate synthetic routes to U-1 and F-1. The superoxide anion may possibly yield F-1 directly.

PUBLICATIONS

McKinney, J. D., Palaszek, S. M. dePaul, Matthews, H. B., and Mehendale, H. M.: Stereochemical aspects of the metabolism and toxicity of some chlorinated polycyclodiene pesticide systems. Industrial Medicine and Surgery, July, 1973 (in press).

Serial No.: NIEHS-AST-039
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Dieldrin and Related Polycyclodiene Pesticides as Model Systems for Chemical Study of Enzyme Reaction Mechanisms - Oxidative and Hydrolytic Type"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-032

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: S. M. dePaul Palaszek, Ph.D., H. B. Matthews, Ph.D., and H. Mehendale, Ph.D.

COOPERATING UNITS: Pharmacology-Toxicology Branch

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To extend our knowledge of certain oxidative enzyme reaction mechanisms facilitated by the unique and rigid chemical structure and biological stability of dieldrin and other related chlorinated polycyclodiene pesticides, their metabolites and derivatives. To establish the utility of these systems as model compounds for the study of differential binding properties and metabolism of certain steroid hormones and their precursors.

METHODS EMPLOYED: A variety of synthetic, chromatographic, autoradiographic, and spectroscopic techniques including proton magnetic resonance and chemical ionization mass spectrometry.

MAJOR FINDINGS: Our studies have dealt primarily with the major metabolites of dieldrin excreted by the rat, trans-4,5-dihydroxy-455-dihydroaldrin and the major fecal (C-12 ketophotodieldrin) metabolites. Both chemical and biological studies support our prediction that dieldrin may be initially metabolized to a cis-diol which in turn is epimerized to the trans. In addition, there is biological evidence that the epimerization reaction probably involves an intermediary α-hydroxy ketone which is nonstereoselectively metabolized to both isomeric diols of which the cis isomer recycles. An nmr study has confirmed that the net result of the epimerization process is stereospecific because the trans product has optical activity. Optical activity has been previously observed for the trans-diol product of

dieldrin metabolism. The enzyme system possesses several characteristics typical of mixed-function oxidases but an anaerobic atmosphere had no effect on the reaction.

Chemical studies have shown that association (Lewis acid type) of the epoxide oxygen of dieldrin can sufficiently activate the C-12 methylene bridge which in turn can transmit this activation onto the dichloroethylene bridge. Therefore, such an association with an enzyme could afford the major fecal metabolite as well as the major urinary metabolite via a photodieldrin type intermediate. However, the dichloroethylene double bond is not unreactive under chemical conditions which model certain oxygenase enzyme reactions. Therefore, the possibility that the urinary metabolite is formed via direct attack on the chlorinated double bond cannot be ruled out at this time. No substantial evidence could be obtained to support a hydride abstraction (at C-12) mechanism for generating a common intermediate for the major fecal and urinary metabolites. The fact that very little of the urinary metabolite is excreted by female rats suggests that the female has difficulty in oxidatively dechlorinating the suspected photodieldrin type system. This sex difference may be linked to a male steroid hormone metabolizing enzyme. Complementary metabolism studies of aldrin in plants have indicated that a series of related polar metabolites are formed which appear to be largely the result of the action of one enzyme system. The products can be reasonably explained by an oxenoid mechanism. The apparent stimulation of the enzyme by NADPH and the formation of a product which could be the result of a NIH shift suggest a resemblance to the mixed-function oxidase type systems found in mammals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated polycyclodiene pesticides are among the most stable and persistent insecticides that can contaminate our environment. Their continued use necessitates the elucidation of their metabolic pathways in mammalian systems. A great deal of insight into organic reaction mechanisms has been obtained via the study of bridged polycyclic compounds such as dieldrin. Perhaps greater biological significance will be derived from the use of these systems as model compounds for studying enzyme reaction mechanisms as well. All of the enzymatic reactions being investigated for dieldrin and aldrin have their analogies in steroid metabolism.

PROPOSED COURSE: To further elaborate the stereospecific nature of the biological epimerization of the cis-aldrindiol to the trans. To measure where possible the toxicological properties of the intermediary systems which may occur in the epoxide hydration of dieldrin. To further establish cis-hydration as a significant process in the hydration of certain sterically constrained epoxides such as dieldrin. To investigate a possible biological antagonism between dieldrin systems and certain male or female hormones. To delineate the role of metabolite conjugates in dieldrin metabolism as a model system.

PUBLICATIONS

McKinney, J. D., Palaszek, S. M. dePaul, Matthews, H. B., and Mehendale, H. M.: Stereochemical aspects of the metabolism and toxicity of some chlorinated polycyclodiene pesticide systems. For presentation at the 8th Interamerican Conference on Toxicology and Occupational Medicine, Miami, Fla., July, 1973.

McKinney, J. B., Matthews, H. B., and Wilson, N. K.: Optical purity determination of a pesticide metabolite epimerization product with a chiral nmr shift reagent. Submitted to Tetrahedron Letters, 1973.

Matthews, H. B., and McKinney, J. D.: Cis-diol formation in the epoxide. Hydration of dieldrin. (in preparation) 1973.

McKinney, J. D., and Mehendale, H. M.: Aldrin metabolism by pea and bean root preparations. Formation of polar metabolites. Submitted to J. Agric. Food Chem., 1973.

Serial No.: NIEHS-AST-040
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "DDT and Related Compounds as Model Systems for Chemical Study of Enzyme Reaction Mechanisms-Dehydrochlorination, Reductive Dechlorination, and Oxidation"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-033

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: S. M. dePaul Palaszek, Ph.D., N. Chopra, Ph.D., P. Chen, Ph.D., and C. Ford

COOPERATING UNITS: Department of Chemistry, N. C., A&T State University, Greensboro, N. C.

MAN YEARS

Total:	0.30
Professional:	0.25
Other:	0.05

PROJECT DESCRIPTION

OBJECTIVES: To investigate model chemical hydroxylation of the DDT benzhydryl position and the aromatic ring. To investigate oxidation and conjugative reduction of various saturated DDT metabolites as a mechanistic alternative to dehydrochlorination. To investigate the products of reductive dechlorination of saturated DDT metabolites and oxidative dechlorination of unsaturated metabolites.

METHODS EMPLOYED: A variety of synthetic, chromatographic and spectroscopic techniques.

MAJOR FINDINGS: Reductive dechlorination of acetoxy DDT (kelthone acetate) resulted in high yields of DDE suggesting that oxidation followed by conjugative reduction could be an alternative mechanism for the formation of unsaturated DDT metabolites as opposed to the DDT dehydrochlorinase reaction. In addition, similar conjugates, if formed in biological systems, could function as reasonably strong oxidizing agents which could interact with reduced porphyrin systems. Such a mechanism is consistent with the *in vivo* metabolism of kelthane to DDE. Other chemical studies have shown that the proposed acetaldehyde precursor of DDA is rapidly converted to the 4,4'-dichlorobenzophenone in the presence of biologically significant amino containing compounds. In addition,

attempted in vitro conversion of the aldehyde to DDA resulted in almost total degradation to the benzophenone even in the absence of enzyme. The aldehyde apparently can function as a one carbon donor by a mechanism which at present remains unclear. In addition, it is known that the hydrated aldehyde (glycol derivative) can function as an alkylating reagent. Therefore, it seems unlikely that the aldehyde is the true biological precursor of DDA. A reasonable alternative pathway to DDA would be oxidative dechlorination of DDE which may be a common detoxifying mechanism for several chlorinated hydrocarbons.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The broad range of biological activity observed for DDT makes it an attractive model system for complementary chemical investigations. A knowledge of the basic chemistry of DDT should provide a basis for studying the interactions of DDT in biological systems on a molecular level, i.e., enzyme interactions, charge transfer complexation, etc., such that metabolism as well as storage propensities of DDT and similar halogenated xenobiotics can be better understood.

PROPOSED COURSE: To subject both the unsaturated and saturated DDT systems to model hydroxylating conditions known to mimic biological oxidations. To subject the oxidized products and their chemical conjugates to reductive dechlorinating conditions. To determine the relative degree of one-carbon donor ability, oxidizing ability, and alkylating ability of the various oxidative and reductive products from above.

Serial No.: NIEHS-AST-041
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Utilization of Rare Earth Metals in the Analysis of Environmental Agents"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-044

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATOR: N. K. Wilson, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To test the complexing ability of complexes of rare earth metals, europium and praseodymium, for the selective complexation of impurities in the analysis of diverse environmental agents and their metabolites from various biological fluids and tissues. (2) To characterize the major metabolites of the DDT and chlorinated polycyclodiene type pesticides with both chiral and achiral NMR selective shift reagents with particular emphasis on assessing stereochemistry. (3) To demonstrate the utility of NMR systems as instruments of prediction as well as measurement in respect to the chemistry of these chlorinated pesticides.

METHODS EMPLOYED: A variety of synthetic, chromatographic, and spectroscopic techniques, in particular electron impact and chemical ionization mass spectrometry, gas liquid chromatography and nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: Rare earth metal complexes of europium have been utilized for the selective complexation of impurities in the GC/MS analysis of silylation products of the hydroxylic metabolites of the chlorinated polycyclodiene pesticides. Both chiral and achiral NMR selective shift reagents of rare earth metal types have been used to assess the stereochemistry of various chlorinated polycyclodiene oxidative metabolites. Such studies have revealed the stereoselective as well as the stereospecific nature of the enzyme reactions responsible for their formation.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One must know the exact identities of the chemical constituents of our environment before one can begin to accurately assess their potential as hazardous environmental contaminants. Selective complexation of impurities in biological extracts would allow rapid detection of much lower concentrations of metabolites without laborious prior clean-up procedures. The use of rare earth shift reagents coupled with nuclear magnetic resonance spectroscopy enables one to more thoroughly establish the structures of potentially hazardous polyfunctional environmental agents.

PROPOSED COURSE: To seek more selective rare earth complexing agents which may be applied to various biological systems in order to facilitate isolation and characterization of metabolites.

PUBLICATIONS

McKinney, J. D., Fishbein, L., and Barling, L.: Selective complexation of impurities in the EC-GC determination of some chlorinated polycyclodiene pesticide metabolites and derivatives. Bull. Environ. Contam. Toxicol. 7(1): 2, 1972.

McKinney, J. D., Wilson, N. K., Keith, L. H., and Alford, A. L.: NMR characterization of some chlorinated hydrocarbon pesticides, their metabolites, derivatives and complexes. (Presented at the 165th ACS National Meeting, Dallas, Texas, April, 1973).

Serial No.: NIEHS-AST-042
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism and Health Effects of Ingested Hydrocarbons"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-009

PRINCIPAL INVESTIGATOR: Phillip W. Albro, Ph.D.

OTHER INVESTIGATOR: R. Thomas

COOPERATING UNITS: None

MAN YEARS

Total:	0.465
Professional:	0.465
Other:	0.000

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate the relationship(s) of accumulation of dietary paraffins in animal tissues to such suspected pathologic effects as lipidoses, co-carcinogenesis and interference with reticulo-endothelial functions. (2) To determine the specificities of various animal tissues for the accumulation of individual hydrocarbons from defined diets. (3) To elucidate the pathway for catabolism of ingested hydrocarbons, particularly with respect to interference by drugs, pesticides, and synergists. (4) To determine to what extent hydrocarbons may be used as model compounds in predicting metabolic modification of aliphatic chains in other classes of compounds.

METHODS EMPLOYED: The basic test organism is the rat, to which hydrocarbons are administered orally in corn oil, mixed with rat chow, injected I.P., I.V. or I.M. In some cases radioactive tracers (1-^{14}C-hexadecane, 1-^{14}C-octadecane, and U-^{14}C-phytane and 2,3-^{3}H-phytane synthesized here) are used in metabolic studies. Standard chromatographic, immunologic, radioassay and other biochemical techniques are used, except that balance studies of absorption from the gut are made as described in Biochim. Biophys. Acta 219: 437-446, 1970.

MAJOR FINDINGS: (1) The increased sensitivity of pristane-fed rats toward egg albumin-induced anaphylaxis and gross visceral adhesions appears to involve changes in capillary permeability and in other membrane properties. The causative agent may be a metabolite of pristane. (2) Phytane is metabolized by rats to a series of water-soluble fatty acids which are excreted in the urine and to a tertiary alcohol which accumulates in liver and kidneys. The

water-soluble acids, besides serving as lipid precursors in the rat, offer clues as to the general catabolic pathway. (3) Hydrocarbons substituted with a benzene ring are absorbed in the gut of rats more readily than are the corresponding cyclohexane-substituted hydrocarbons.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ingestion of paraffins from a variety of sources and its accumulation (as well as that of other hydrocarbons) in human tissues in cases of lipoid pneumonia, follicular lipidosis of the spleen and meningioma all suggested that tissue accumulation of hydrocarbons is undesirable. Investigations are in progress to elaborate the parameters involved in access of hydrocarbons to the tissues. The most recent findings suggest a previously unsuspected pathway for oxidation of alkyl chains that may have general significance. Because mineral oil, rich in branched alkanes, is a nearly ubiquitous food additive and is on the GRAS list, the recent findings of an extremely undesirable effect of pristane (a branched alkane) on reticuloendothelial function in rats suggest that ingestion of mineral oil deserves careful study.

PROPOSED COURSE: An attempt will be made to obtain sufficient 2,6,10,14-tetramethylhexadecan-2-ol and/or 2,6,10,14-tetramethylpentadecan-2-ol for feeding studies in order to determine whether they are implicated in the hypersensitization properties of pristane and whether detailed toxicological studies are likely to be fruitful. The study of the catabolic pathway for phytane will be continued, and *in vitro* studies will be emphasized in an attempt to identify the early intermediates of its breakdown. Techniques will be refined in order to carry out more informative studies on the nature of the hypersensitizing properties of pristane. The mechanism of action of inspired mineral oil in causing lipoid pneumonia will receive study. The studies made involving pristane will be extended to include commercial mineral oil.

PUBLICATIONS

Albro, P. W., Thomas, R. O., and Fishbein, L.: Increased sensitivity to egg albumin in rats fed pristane. J. Allergy and Clin. Immunol., 1973 (in press).

Serial No.: NIEHS-AST-043
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Transport and Metabolism of Phthalate Esters"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-046

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: L. Fishbein, Ph.D.; R. Thomas

COOPERATING UNITS: None

MAN YEARS

Total:	0.605
Professional:	0.605
Other:	0.000

PROJECT DESCRIPTION

OBJECTIVES: To prepare purified standard labeled and unlabeled phthalate esters, e.g., DHP [di(2-ethylhexyl)phthalate] and DOP (dioctyl phthalates); and to elaborate aspects of their transport, absorption, excretion and tissue residues following various routes of administration, e.g., oral, inhalation and i.p., in the rat.

METHODS EMPLOYED: Chromatography (TLC, column, GLC), spectrophotometry (I.R. U.V., visible), mass spectrometry and isotopic methods, standard enzymology techniques.

MAJOR FINDINGS: (1) The urinary metabolites of di-(2-ethylhexyl)phthalate in the rat have been identified. The metabolites give a clear reflection of the metabolic pathways involved (see Publications). (2) The resistance of diethylhexyl phthalate to hydrolysis or metabolism by rat intestinal microflora has been confirmed. (3) The rate of absorption of diethylhexyl phthalate in rat gut is essentially zero, due to very rapid lipolytic hydrolysis. The hydrolytic products, however, are readily absorbed. (4) The susceptibility of diethylhexyl phthalate and of monoethylhexyl phthalate to hydrolysis by 16 tissue lipases of the rat, and intestinal lipases of guinea pig, hamster, and mouse has been determined (see Publications). (5) Phthalate esters are being used as advantageous substrates for the isolation and characterization of "non-specific lipase" and other lipases acting on micellar substrates.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed extensively,

primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the enzyme consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

PROPOSED COURSE: (1) Studies made using diethylhexyl phthalate will be extended to include other phthalate esters. (2) Rat liver "alkaline lipase", a microsomal enzyme, and rat pancreas "nonspecific lipase", a soluble enzyme, differ considerably as to the physical state of substrate they prefer. Because many of the more prominant environmental agents are water-soluble, the interactions of phthalate esters with the above lipases should provide generally useful data on the physical interactions of soluble and insoluble enzyme systems with insoluble substrates. This will be studied in detail. (3) The metabolism of the alcohol moiety of diethylhexyl phthalate will be investigated.

PUBLICATIONS

Albro, P. W., Thomas, R., and Fishbein, L.: Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr., 1973 (in press).

Albro, P. W., and Thomas, R. O.: Enzymatic hydrolysis of di-(2-ethylhexyl)-phthalate by lipases. Biochim. Biophys. Acta, 1973 (in press).

Serial No.: NIEHS-AST-044
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Chemistry of Substituted Allyl and Propenyl Benzenes and Their Proposed Metabolites Including Synthesis of Radioactive Counterparts"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-030

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D., M. Walker, and C. Ford

COOPERATING UNITS: None

MAN YEARS

Total:	0.7
Professional:	0.1
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To investigate chemical model oxidizing conditions for allyl and propenyl benzenes that could afford vinyl ketones or allyl oxides as reactive intermediary systems. To synthesize radioactive counterparts for the natural products themselves as well as their potential metabolites for diverse metabolism and toxicological studies.

METHODS EMPLOYED: A variety of synthetic, chromatographic and spectroscopic techniques.

MAJOR FINDINGS: The synthesis of 3,4-methylenedioxyphenyl acetaldehyde, a Wittig reaction precursor of safrole, has been accomplished via degradation of commercially available safrole. A modified Wittig reaction has been developed which enables reformation of the allyl group affording safrole in reproducibly good yields. The methodology developed should have general applicability for positioning of a radioactive label (^{14}C) in the terminal carbon of the allyl side chain for all the natural products of interest which can be obtained in reasonably pure form. Selective dealkylation and reformation of the methylenedioxy ring offers promise as a synthetic route to some of the natural products that are not available. Still other synthetic methods are available for preparing the desired acetaldehyde derivative from the corresponding toluenes. This latter approach will permit maximum variability in positioning of radioactive labels. The development of these synthetic methods for safrole has revealed the unusually sensitive nature of the methylenedioxy ring system

under a variety of conditions which may have biological consequence.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The substituted allyl and propenyl benzenes are widely distributed in nature and some are known to be pesticidal synergists. Therefore, it is necessary to isolate, quantitate, and identify their metabolites and other conversion products to ascertain whether or not exposure to or ingestion of these agents constitutes a potential environmental hazard. An understanding of the fundamental chemistry of these systems and the utility of their radioactive counterparts should facilitate their localization and characterization in biological and chemical studies.

PROPOSED COURSE: To synthesize safrole labeled in the terminal carbon of the allyl side chain utilizing the methods developed. To prepare in a similar manner the acetaldehyde Wittig reaction precursors of myristicin and elimicin. To investigate the oxidation of safrole under hydride abstracting conditions. To develop methodology for determining the relative degree of the acylethyl alkylating ability of the suspected vinyl ketone metabolic intermediates of safrole, myristicin, and elimicin.

Serial No.: NIEHS-AST-045
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Biosynthesis of Piperidine and Pyrrolidine"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-031

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: J. D. Peele, B. J. Corbett

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.1
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: Allyl benzene metabolic derivatives are linked biologically to tertiary amino propiophenones, e.g., the amine condensing units of each ketone are dimethylamine, piperidine and pyrrolidine. Very little information is available on the biosynthesis of these particular secondary amines. It is very desirable to determine the biological sequence of events that yield dimethylamine, and especially piperidine and pyrrolidine. Piperidine and pyrrolidine possess "nicotine-like" properties in biological systems. In addition, the correlation of psychoactive properties of allyl benzene metabolites of essential oil with that of these secondary amines should enhance the evaluation of potential hazard of these materials as environmental agents.

Because of the production of the carcinogenic nitrosamines of piperidine and pyrrolidine in foods and other biological systems, a thorough evaluation of these secondary amines is greatly needed.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), cell fractionation by ultracentrifugation, enzyme analysis, and radioactive tracer metabolism followed by NMR, IR and mass spectrometry.

MAJOR FINDINGS: Methods of analysis of piperidine and pyrrolidine have been evaluated and developed for the quantitative and qualitative analysis of these secondary amines in biological systems including human urine and ratbbrain. Dinitrophenyl derivatives of piperidine and pyrrolidine have been synthesized and characterized by TLC, GLC, NMR, IR, visible spectroscopy, and mass spectrometry. Spectrophotometric analyses of the dinitrophenyl derivatives of

piperidine and pyrrolidine indicate linearity over a range of 0.5 to 7 µg/ml. Recovery of oxogeneous piperidine from human urine ranges from 26% to 42%. Recovery of oxogeneous piperidine from rat brain supernatant ranges from 50-54%.

Preliminary investigations have indicated that the DNP amine can be detected in picogram quantities using gas chromatography with an electron capture detector. Because of the desirable detectable limits of DNP-piperidine (at least 10 picograms) by electron capture, this method of analysis appears most promising for quantitation of low concentrations of piperidine and pyrrolidine in biological tissues. In addition, this gas chromatographic analysis eliminates previously required TLC separation and elution, therefore, minimizing manipulations which greatly decreases the recovery of these amine derivatives.

Because of the very low decarboxylase activity with proline and pipecolic acid, investigations are underway to study the enzymatic production of piperidine and pyrrolidine from ^{14}C substrates.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding and knowledge of the rates of production and elimination of piperidine and pyrrolidine are essential in determining the potential of these biological materials as health hazards.

PROPOSED COURSE: Characterize and purify the decarboxylase systems responsible for the conversion of proline and pipecolic acid to pyrrolidine and piperidine, respectively. Characterize the formation of piperidine and pyrrolidine from ^{14}C labeled glutamic acid and ^{14}C labeled lysine using the rat, guinea pig and some level of primates. Investigate the bacterial decarboxylase responsible for formation of piperidine and pyrrolidine.

Serial No.: NIEHS-AST-046
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Allyl and Propenyl Benzenes"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-003

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATOR: B. Corbett

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.8
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To identify the various urinary metabolites of myristicin, safrole, isosafrole, elemicin, eugenol, and asarone, following administration by various routes to the rat and other biological test systems. (2) To investigate the general metabolism of these allyl and propenyl benzenes with radiolabeled substrates. (3) To coordinate this chemical and metabolic information so that one may determine the potential hazards produced by these natural product environmental agents.

METHODS EMPLOYED: Chromatography (TLC, column, and GLC), NMR, IR and mass spectroscopy, and standard radioisotopic analyses.

MAJOR FINDINGS: Following oral or intraperitoneal administration of myristicin, safrole, isosafrole, elemicin, eugenol, asarone (trans) or beta asarone (cis) to male rats, basic ninhydrin positive materials are excreted in the urine. There is an apparent requirement of a side chain double bond for the production of these materials with greater enhancement by the trans isomer than the cis.

Complete structural identification of the ninhydrin positive metabolites of safrole, myristicin, elemicin and eugenol indicates that the nitrogen containing metabolites are tertiary amino propiophenones. The amine substituents of these tertiary amine propiophenones excreted by the rat and guinea pig are: (I) dimethylamino, (II) piperidine, and (III) pyrrolidine. Depending upon the structure of the administered allylbenzene and the specific test species, varying abundances of these specific amine substituted tertiary aminopropiophenones were noted. The piperidine substituted metabolites seem to be the

major urinary excretion metabolites of the allylbenzenes examined to date.

Initial metabolic investigations with methylenedioxy ^{14}C labeled safrole indicate that approximately 50% of the orally administered ^{14}C is released as $^{14}CO_2$ within 27 hours in the rat. Feces and urine account for about 20% of the label during the 27 hour period. Analysis of biological samples at varying time intervals indicates that the distribution of ^{14}C derived from methylenedioxy ^{14}C labeled safrole was general but not equally distributed throughout the rat. The liver contained the greatest amount of radioactivity for all time periods with the small intestine containing the second most concentrated quantity of label.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Unequivocal identification of the metabolites and the knowledge of the rates of elimination and degree of storage of these environmental agents are essential in determining the potential health hazards produced by exposure and injection of the allyl and propenyl benzenes and related compounds.

PROPOSED COURSE: (a) To complete the identification and characterization of the ninhydrin positive metabolites of isosafrole and the asarones. (b) To prepare ^{14}C labeled myristicin, safrole, elemicin and eugenol. (c) To complete the general metabolism of the ^{14}C labeled allyl and propenyl benzenes in whole animal and cell free systems.

PUBLICATIONS

Oswald, E. O., Fishbein, L., Corbett, B. J., and Walker, M. P.: Metabolism of naturally occurring propenyl benzene derivatives. II. Separation and identification of tertiary aminopropiophenones by combined gas-liquid chromatography and chemical ionization mass spectrometry. J. Chromatogr. 73: 43-57, 1972.

Oswald, E. O., Fishbein, L., Corbett, B. J., and Walker, M. P.: Chemical lability of the tertiary aminopropiophenones of eugenol as characterized by combined gas-liquid chromatography and chemical ionization mass spectrometry. J. Chromatogr. 73: 59-72, 1972.

Serial No.: NIEHS-AST-047
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolic Interaction of Amines and Tertiary Amino Propiophenones with Monoamine Oxidase Systems"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-029

PRINCIPAL INVESTIGATOR: V. S. Bangdiwala, Ph.D.

OTHER INVESTIGATOR: E. O. Oswald, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To characterize the interaction and inhibition of the nitrogen metabolites of the allyl benzenes - namely the tertiary amino propiophenones and specific amines with mitochondrial monoamine oxidase systems.

METHODS EMPLOYED: U.V.-Visible Spectroscopy, cellular fractionation by ultracentrifugation, electron microscopy, and standard enzyme analyses.

MAJOR FINDINGS: Comparative studies indicate that tissues from young rats (100-150g) provided a much more desirable source of monoamine oxidase than those of older rats (350-500g). The purity and recovery of mitochondria from tissue homogenate (1:10) was determined by analysis of the enzyme maker system - cytochrome C oxidase along with monoamine oxidase and by electron microscopy. Approximately 60-70% of the monoamine oxidase activity was located in the mitochondria with 30-40% of the enzyme activity residing in the nuclear fraction. Little or no monoamine oxidase activity was found in the final supernatant fraction.

Preliminary inhibition studies were made with piperidine, pyrrolidine, the piperidine metabolite (II) of elemicin and the pyrrolidine metabolite (III) of safrole. The amines and tertiary amine propiophenones of elemicin and safrole exhibited varying degrees of inhibition dependent upon the tissue and the specific substrate. For example, piperidine and pyrrolidine with serotonin as substrate did not inhibit liver, brain or kidney mitochondrial monoamine oxidase; on the contrary, with tyramine as substrate piperidine inhibited

mitochondrial monoamine oxidase preparation of all three tissues 40-100%.

The tertiary amino propiophenone metabolites of elemicin (II) and safrole (III) both inhibited liver, brain, and kidney monoamine oxidase systems with both tyramine and serotonin substrate at levels varying from 60-100% inhibition.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough understanding of the interaction of the tertiary aminopropiophenones and specific amines with mitochondrial monoamine oxidase systems would help to remove the existing ambiguities related with the psychoactive properties of various components of essential oils. This information would furnish a better means of evaluation of these environmental agents as potential health hazards.

PROPOSED COURSE: (1) Complete inhibition studies with the tertiary amino-propiophenones and specific amines using mitochondrial preparations. (2) Solubilize and purify the most desirable monoamine oxidase system. (3) Compare inhibition studies upon purification with those of the crude enzyme system. (4) Characterize and identify any metabolite of the tertiary amino-propiophenones formed during enzyme incubations.

PUBLICATIONS

Oswald, E. O., Fishbein, L., Corbett, B. J., and Walker, M. P.: Metabolism of naturally occurring propenyl benzene derivatives. II. Separation and identification of tertiary aminopropiophenones by combined gas-liquid chromatography and chemical ionization mass spectrometry. J. Chromatogr. 73: 43-57, 1972.

Serial No.: NIEHS-AST-048
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "NMR Investigations of Structures and Molecular Interactions of Nitrosamines"

PREVIOUS SERIAL NUMBER:

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATOR: J. B. Stothers, Ph.D. (University of Western Ontario)

COOPERATING UNITS: University of Western Ontario

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the detailed molecular geometry and electronic structures of aliphatic and aromatic nitrosamines; to study their molecular interactions with models for biologically significant systems, and to determine the possible means of their formation and decomposition in such model systems.

METHODS EMPLOYED: Variable temperature nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: ^{13}C nuclear magnetic resonance data have been obtained for several simple aliphatic nitrosamines.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nitrosamines are known potent carcinogens; they have been found to occur in certain foods, and additionally, they may possibly be formed in the gastro-intestinal tract from nitrites and nitrates in foods. Thus knowledge of their molecular properties and chemistry is of great importance in evaluation of the present and potential hazards due to this class of compounds.

PROPOSED COURSE: The temperature dependence of the magnetic resonance parameters for the simple aliphatic nitrosamines will be determined, to enable extraction of the thermodynamic and activation parameters governing their possible conformational interconversions. Extension to some aromatic nitrosamines is planned.

Serial No.: NIEHS-AST-049
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "^{13}C NMR Studies of Binding in Aromatic Carcinogens"

PREVIOUS SERIAL NUMBER:

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To obtain and analyze the ^{13}C nmr spectra of polynuclear aromatic carcinogens, and to investigate the effect of possible complexing agents on these spectra and thus illuminate the possible modes of interaction of the aromatic carcinogens in biological systems.

METHODS EMPLOYED: Variable temperature nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: Preliminary data has been obtained.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although work on carcinogenic compounds of this type has been extensive, and the hazards associated with their production and use are well-documented, their mode of interaction in biological systems is not fully understood. The studies being conducted here may help to elucidate the mechanisms of carcinogenicity of these compounds.

PROPOSED COURSE: ^{13}C magnetic resonance spectra of some simple compounds of this type will be obtained and analyzed. The effect of various possible complexing agents on these spectra will be determined, and if possible, the equilibrium thermodynamic parameters will be extracted from the data.

Serial No.: NIEHS-AST-050
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "NMR Investigations of Metallic Linear and Cyclic Dithiocarbamates"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine activation and thermodynamic parameters for the decomposition of a variety of cyclic and linear metallodithiocarbamates and to investigate the molecular and electronic structures of these compounds.

METHODS EMPLOYED: Variable temperature nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: Preliminary ^{13}C nmr data on several cyclic dithiocarbamates has been obtained.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The cyclic and linear metallodithiocarbamates have wide utility as fungicides and rubber accelerators and, hence, are of environmental significance. Ethyl selenac has also been shown to be an effective enzyme inhibitor. Elaboration of the molecular structures of these compounds, possible decomposition products and their rates of formation may illuminate both the mode of inhibition and the possible environmental hazards associated with the use of dithiocarbamates.

PROPOSED COURSE: Continue this work, with extension to other metallic dithiocarbamates.

PUBLICATIONS

Wilson, N. K., and Fishbein, L.: Decomposition of tetrakis(N,N-diethyldithiocarbamato)selenium (IV). J. Agr. Food Chem. 20: 847-850, 1972.

Serial No.: NIEHS-AST-051
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Prostaglandins as Environmental Factors"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-021

PRINCIPAL INVESTIGATOR: Phillip W. Albro, Ph.D.

OTHER INVESTIGATOR: R. Thomas

COOPERATING UNITS: None

MAN YEARS

Total:	0.46
Professional:	0.46
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: (1) Development of a highly sensitive, specific analytical procedure for qualitative and quantitative determination of prostaglandins. (2) Evaluation as to whether exogenous prostaglandins should be considered environmental health factors, either relative to ingestion or topical exposure. (3) Determination as to whether endogenous prostaglandins play a significant role in the response of animals to drugs, pesticides, antigens and the like.

METHODS EMPLOYED: (1) A procedure has been developed by which prostaglandins may be assayed by gas chromatography (J. Chromatog. 44: 443, 1969). A two-dimensional thin-layer chromatographic technique involving specific visualization reagents is under development for qualitative detection of prostaglandins. A procedure for extraction and purification of prostaglandins to be used as a preliminary to GLC and TLC has been developed which is suitable for screening purposes but which does not give adequate recoveries for quantitative assays. (2) A technique for studying the effects of prostaglandins on mast cells in vitro has been developed (Prostaglandins 1: 133-146, 1972).

MAJOR FINDINGS: (1) Prostaglandins E_1 but not $F1_\alpha$ stimulates the release of histamine by rabbit anti-rat gamma globulin from rat peritoneal cells. Stimulation occurs between 25 nanograms PGE_1 per ml to 1.5 µg/ml PGE_1, but levels of PGE_1 between 3 and 30 µg/ml inhibit antibody-induced histamine release. Thus E-type prostaglandins may partially sensitize or desensitize rats to antibody-induced histamine-release, depending on local PGE levels. (2) Some evidence has been obtained suggesting that PGA and PGE but not $PGF1_\alpha$ may stimulate diamine oxidase (Histaminase) activity in vitro. These experiments

are incomplete.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because continuing review of the literature indicates that many of the properties of prostaglandins mentioned under "Objectives" above are currently under investigation in a number of laboratories, we have presently concentrated on the role of prostaglandins in the anaphylactic response as being most directly related to their possible environmental significance, but not presently under (saturating) study elsewhere. Our results to date suggest more of a protective than a harmful role for prostaglandins in anaphylaxis.

PROPOSED COURSE: (1) We still wish to make studies of the effects of orally administered prostaglandins on laboratory animals, but this will have to wait until gram quantities of prostaglandins become available. (2) We will complete our studies on the effects of prostaglandins on sensitized mast cells as soon as the technical difficulties in obtaining sensitized cells are overcome. A new technique for obtaining respiratory tissue mast cells appears promising (Science 195: 1012, 1972), and will be considered.

Serial No.: NIEHS-AST-052
1. Animal Science and Technology Branch
2. Chemistry Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of Chronic Exposure of Heavy Metals on Rats"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-022

PRINCIPAL INVESTIGATOR: M. D. Folsom, Ph.D.

OTHER INVESTIGATOR: John Fawkes

COOPERATING UNITS: None

MAN YEARS

Total:	1.25
Professional:	.75
Other:	.50

PROJECT DESCRIPTION

OBJECTIVES: To determine the biochemical effects of longterm exposure to low levels of heavy metals, particularly as effects on enzyme systems, as correlated with storage and with other symptoms of toxicity.

METHODS EMPLOYED: Atomic absorption spectrometry, gas chromatography, enzyme assays, histological studies.

MAJOR FINDINGS: Administration of a total of 31 mg/Kg of CH_3Hg^+ and Hg^{++} over a twelve week period showed greater storage of methyl Hg in all organs studied (blood, liver, brain, lung, spleen) with the exception of the kidney, where methyl and inorganic Hg were stored in equal amounts (total Hg). Distribution among subcellular fractions of kidney showed greatest storage in the nuclear fraction (50% Hg:30% CH_3Hg) with second highest levels in the cytoplasmic fraction (20% both groups). Mitochondria contained 15% Hg:20% CH_3Hg treated. Microsomes contained 15% in Hg treated, 19% in CH_3Hg treated. Most of the mercury was stored as CH_3Hg in the methyl Hg treated animals; however, the percentage of inorganic Hg increased with time after exposure was discontinued.

Effects of inorganic and organic Hg on kidney enzymes were highly significant. Organic and inorganic Hg had similar effects, but differed in degree. In most instances effect of inorganic Hg was greater (glutamate dehydrogenase, carbonic anhydrase, alkaline and acid phosphatase). Lactate dehydrogenase activity was increased by inorganic Hg and unaffected by methyl Hg.

In vitro studies with corresponding amounts of metal incubated with kidney preparation showed inhibition, presumably by combination with SH groups,

indicating that the in vivo effects are due to more widespread response. Cellular damage, and a pronounced proteinuria, were further evidence of kidney toxicity. Cadmium exposure (3 mg/kg/week) resulted in increased proteinuria as early as six weeks after initiation of exposure. Activity of kidney enzymes was significantly increased similar to, but somewhat less than, the increases produced with exposure to equivalent amounts of inorganic Hg. In vitro experiments also showed inhibition of enzymes of kidney preparations incubated with corresponding amounts of Cd.

Effects on kidney enzymes of arsenic have been measured. Activities of alkaline phosphatase, acid phosphatase, and carbamic anhydrase were increased with arsenite. Catalase was unaffected by arsenate, but was increased by arsenite. Arsenate produced an inhibition of alkaline phosphatase, acid phosphatase, and esterase. For all metals studied, males are apparently more susceptible than the females.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Wide use of metals in industrial products and processes makes it essential to understand the biological effects of prolonged exposure to various levels of metals, in order to determine the nature of the hazard to man.

PROPOSED COURSE: Studies are now in progress to further study the relationship of enzymatic changes to histological damage. Additional enzymes are being investigated, as are excretion patterns.

PUBLICATIONS

Folsom, M., Fishbein, L.: Effects of repeated sub-lethal dosages of methyl mercury in the rat. Sci. Total Environ. 1: 91-95, 1972.

BIOMETRY BRANCH

BIOMETRY BRANCH
Summary Statement

The Biometry Branch fulfills a dual mission, functioning both in a service capacity to the intramural program, and also as an applied research group. Statistical support for the Institute is provided mainly for the design of experiments and in the analysis of laboratory data. Data processing and computer programming services are available to the research staff of the Institute and are routinely employed by the Biometry Branch in its statistical applications work.

Research activity is currently being conducted in the general areas of statistical methodology, biomathematics and epidemiology. The statistical research involves both the development of methodology generated from data analysis needs, and the investigation of topics such as clinical trial design and low-dose extrapolation, which are of abiding interest to NIH. Biomathematics research is primarily collaborative, being focused mainly on the mathematical modeling of biological processes. Finally, the epidemiological work being done is concerned with the statistical analysis of existing data bases, the emphasis being on an attempt to detect environmental components and quantify their implications upon human health.

The following breakdown gives an indication of the specific areas within the intramural program where statistical applications and data processing services are rendered.

STATISTICAL AND PROGRAMMING SUPPORT

PATHOLOGIC PHYSIOLOGY BRANCH

Investigations are being conducted to determine the most appropriate means of statistically evaluating the toxic effects of environmental chemicals on oogenesis. Particular emphasis is being placed on the inter-relationships of various parameters of reproductive capacity, such as the number of litters, total number of offsprings, and approximate period of fertility. The efficacy of nonparametric procedures in dealing with the arbitrary censorship introduced into the experimental data by random deaths is also being considered. Finally, potential sources of variation related to the experimental design, such as cage and male effects, are being assessed by standard techniques.

In an investigation of the dominant lethal and antifertility effect of several different compounds, classical parameters such as pregnancy rates and live litter size were examined. Treated animals were compared with concurrent controls and also with their own pre-treatment values. Because of the discrete nature of the data and the number of comparisons desired, actual as well as nominal error rates were computed to serve as guidelines in interpreting the

significance of the findings. In conjunction with the analyses, the question of the best means of quantifying dominant-lethal effects is being considered.

Blood chemistry data from a large epidemiological study were analyzed in order to determine if the parameters of interest were sex and race dependent. In addition, the effect of cardiovascular medication on serum uric acid concentrations in four race-sex groups was evaluated.

A computing system is being developed for the statistical analysis of teratology data. The system will provide rapid and efficient testing of various hypotheses related to teratological parameters and will also furnish the scientist with a concise permanent record of his experimental data.

Data from a large number of control litters of both rabbits and mice were analyzed in order to determine whether variables such as fetal weight, mortality, anomalies, and stunted fetuses were associated with factors such as litter size, location of the fetus or maternal weight. For these variables, nested Analysis of Variance procedures were employed to determine whether litter-mates were more homogeneous than were fetuses from different litters.

PHARMACOLOGY AND TOXICOLOGY BRANCH

Statistical analyses were performed to determine the effects of several polychlorinated biphenyls (PCB) on the levels of biphenyl, benzphetamine, benzpyrene and microsomal protein in the rabbit liver and lung. The administration of non-PCB related drugs introduced some confounding into the experiment, but in many cases it was possible to estimate and adjust for these effects.

Statistical support was also provided to evaluate the relationship between enzyme activity and dosage level for a number of compounds. By using polynomial regression techniques, the functional form of these relationships was determined. For compounds showing a significant non-linear dose-response, dosage levels producing maximum simulation were estimated. Time trends in the data were also examined.

Statistical support for the aerotoxicology area was directed primarily at the planning and design phases of the laboratory effort, with routine hypothesis testing performed as required. Investigations were conducted to delineate and quantify potential sources of variation in the experimental methodology. The effect on baseline parameters of animal assignment to treatment groups was studied, the relative magnitude of within-animal-pool to between-animal-pool variation was determined, and the measurement (including calibration and normalization) procedures used to determine enzyme activity were assessed.

Various techniques for the quantification of organ damage were also evaluated. An effort was made to define regions of approximate linearity in curves of enzyme activity versus both protein concentration and incubation time. Design support was provided by determining sample sizes required to detect a specified increase in organ weight and organ-to-body weight ratio.

MUTAGENESIS BRANCH

An extensive computer program which was written at Oak Ridge National Laboratory for the processing of Neurospora mutation data is being revised for use at the Division of Computer Research and Technology, NIH. The computer program routinely makes checks on both the consistency and completeness of the experimental data and performs requisite statistical calculations.

In a dominant lethal assay, both single and continuous doses of Methyl methane sulfonate were administered. Besides the determination of usual dose-response relationships, attempts will be made to predict the continuous dose effects from the single dose results. It will then be possible to determine whether continuous administration of total dose results in decreased or increased effects of dominant lethals.

ANIMAL SCIENCE AND TECHNOLOGY BRANCH

Statistical support was provided to the Animal Science and Technology Branch in the investigation of teratogenic effects of hycanthone and its analogue on both mice and rats. Nonparametric test procedures were employed to test for treatment-control differences in the incidence of fetal mortality and anomalies. Underlying dose-response relationships were also examined.

Statistical analyses were employed to assess the effect of weekly administration of PCB's on the blood chemistry and hematology of rabbits. Possible treatment effects, sex differences, time effects, and interactions among these factors were investigated. Treatment-control comparisons of body weight and several other parameters were also made.

Data from a study investigating the effect of dioxin on various organ weights in guinea pigs and rats were analyzed. Treatment-control comparisons were made both with and without adjusting for differences in final body weight. In some cases heteroscedasticity and imbalance in the experimental design required modification of the usual multiple comparison test procedures. Nonparametric methods were employed to test for dose-response relationships. Finally, the effect of dioxin on tuberculin skin reactions in the two species was also examined.

RESEARCH SERVICES BRANCH

Statistical support was provided for two studies conducted by the Biophysics section. The first involved the continuous monitoring of noise levels and other variables in several hospital rooms. In order to determine which variables were primarily responsible for the noise levels, multiple regression procedures were employed.

In the second study, the ototoxicity of methyl mercury was investigated by evaluating the extent of hair cell damage in guinea pigs. In addition to testing for significant dose-response relationships, possible localization of treatment effects was examined. Recommendations concerning the design and analysis of experiments of this type were made.

OFFICE OF THE DIRECTOR

In response to an appeal made to the Office of the Director by the Division of Biological Standards, the Biometry Branch became involved in a re-evaluation of a clinical trial designed to evaluate the efficacy of a particular medicinal compound. Serious flaws were detected in the experimental design, which gave rise to some questions about the reliability of the original findings. At the present time, the data is being reanalyzed by the initial investigators.

As part of an effort to determine the potential presented by an industrial population of pesticide formulators to quantify the risk to human health involved in pesticide exposure, the mortality experience of a hypothetical normal population was simulated on the computer. The simulations indicated that the increase in either general or disease specific mortality would have to exceed all reasonable expectations in order to be detectable with sample size of individuals presumably available for follow-up. Therefore, it was decided to abort the project.

The analysis of the NTA carcinogenesis study was brought to completion with the application of a new statistical procedure to the survival curves for the various dose levels used. This was done in order to assess the significance of the apparent dose-response pattern observed in the experimental data. The relationship was statistically significant, which added to the findings already reported.

RESEARCH ACTIVITIES

STATISTICAL METHODOLOGY

The development of procedures for improved interpretation and understanding of animal survival studies is being continued. The basic approach taken has been to use nonparametric estimates of the probabilistic models which describe the occurrence of specific diseases in a competing risk framework. Particular attention has been focused on methods of dose-response representations. A nonparametric test for determining the presence of a dose-response effect has been developed and studied. Methods are currently being explored for low-dose extrapolation. In addition, procedures which permit early termination of survival studies are being considered.

Work is also being continued on the development of improved clinical trial designs. The approach has entailed minimizing the number of subjects receiving the inferior treatments while at the same time maintaining the ability to make statistically valid inferences about the treatment effects. Procedures have been developed for selecting the best of several treatments when the response time is random. This has involved the creation of Likelihood-bias sampling, which is a generalization of Play-the-Winner sampling often used with instantaneous response designs.

BIOMATHEMATICS

In collaboration with the Pharmacology and Toxicology Branch, steady-state analyses were employed in an attempt to elucidate the mechanism for the accumulation of amines in the isolated perfused lung. Accumulation by the lung of the basic amines which were directly investigated consisted of a multi-component system, which could be resolved into a linear component and a saturable component for the blood concentration range examined. The linear component could not be due to simple diffusion but is probably non-specific binding. Inhibition studies were used to examine the nature of the saturable process and the dependence of the process on the degree of protonation of the nitrogen atom. The non-basic amines which were studied were accumulated to a small extent and the accumulation process behaved as a simple diffusion process.

Also, in collaboration with the Pharmacology and Toxicology Branch, studies with isolated perfused lung and liver systems are being performed in conjunction with intact animal studies. These systems readily lend themselves to the development of pharmacokinetic models for the uptake, storage, and metabolism of chemicals. A better understanding of the mechanisms involved in these processes can be obtained with the use of isolated organs. Information gained, such as tissue-binding blood isotherm, membrane permeability, metabolic rates, and models obtained for biliary excretion from the isolated perfused liver system can be incorporated into the pharmacokinetic model for intact animals.

Probabilistic models for describing the number of mutant cells in a cell colony as a function of time are being studied. Estimates of cell mutation rates based on these models for cell growth have been developed. The estimates take into account both laboratory procedures for the periodic sampling of the cell cultures and the effects of phenotypic delay.

EPIDEMIOLOGY

Data generated from certified fatal deaths occurring in the state of North Carolina between 1947 and 1970 are being analyzed to determine if there is any evidence of time-space clustering among deaths with neural tube defects as an underlying cause. When preliminary analyses indicate possible clustering, more detailed analyses of both fetal and infant deaths will be conducted. If significant evidence of clustering is found, it may be possible to delineate areas of potential high risk which would, in turn, shed some light on the etiology of this disease.

OTHER ACTIVITIES

Dr. J. K. Haseman is a visiting lecturer in the Department of Mathematics at St. Augustine's College, Raleigh. His duties have included teaching probability and statistics to mathematics majors.

Dr. D. G. Hoel is an Adjunct Assistant Professor of Biostatistics in the School of Public Health, University of North Carolina at Chapel Hill, where he lectures in the graduate school. He is a member of the regional (Eastern North America) council of the Biometrics Society and a member of the statistics panel of the

Scientific Advisory Board of the National Center for Toxicological Research. Dr. Hoel is also an Associate Editor of the Journal of Statistical Computation and Simulation and the Journal of the American Statistical Association.

Serial No.: NIEHS-B-004
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Statistical Methodology and Modeling in the Analysis of Teratology Data"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: J. K. Haseman, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D. and T. A. Clemmer

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop a computing system for the statistical analysis of teratology data.

METHODS EMPLOYED: (1) A data format is being devised to permit the concise summarization of all relevant quantitative information from a general class of teratology studies. (2) A computer program is being developed for the calculation of summary statistics and for the testing of assorted hypotheses related to various teratological parameters.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is anticipated that a large volume of teratology data will soon be generated by various NIEHS studies. Therefore, it is necessary to have rapid and efficient methods for the required statistical analyses. In addition, a computerized analysis system has the advantage that the experimenter retains on computer cards a permanent record of his data.

PROPOSED COURSE: (1) Conduct a pilot study to ascertain suitability of data formating and coding systems, and (2) add additional statistical subroutines to the system as the need arises. These will include multiple regression techniques and additional nonparametric test procedures.

Serial No.: NIEHS-B-005
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Clinical Trials and Statistical Selection Procedures"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: D. G. Hoel, Ph.D. and G. H. Weiss, Ph.D. (DCRT)

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop efficient experimental designs for selecting the best of several experimental treatments.

METHODS EMPLOYED: Data from controlled laboratory or clinical trials are assumed to occur sequentially in time where the individual responses are dichotomous. Sequential regions have been developed for deciding when to terminate the experimental and which treatment to select as most effective.

MAJOR FINDINGS: New and improved procedures are continuing to be developed. Procedures which do not assume instantaneous response to treatment have been extended to more than two experimental populations. Likelihood biased sampling, an extension of Play-the-Winner sampling, has also been developed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The emphasis of these sequential designs is to minimize the number of trials on inferior treatments. This has been overlooked in classical clinical trials and is a critical concern when dealing with human populations.

PROPOSED COURSE: Continued improvement in the efficient use of laboratory and clinical trial data is required. Designs which select the treatment with the best 5 year survival rate will be studied.

PUBLICATIONS

Hoel, D. G. and Sobel, M.: Comparisons of sequential procedures for selecting the best binomial population. Proc. Sixth Berkeley Symposium on Prob. Statist. IV: 53-69, 1972.

Hoel, D. G., Sobel, M. and Weiss, G.: A two stage procedure for choosing the better of two binomial populations. Biometrika 59: 317-322, 1972.

Serial No.: NIEHS-B-006
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Probability Models and Competing Risks in the Study of Mortality Data"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop statistical techniques for assessing the effects of a treatment on specific diseases in survival studies.

METHODS EMPLOYED: A probabilistic model has been studied for describing the occurrences of particular diseases in a competing risk framework. Nonparametric techniques are used to estimate the model, giving both the disease incidence and cumulative mortality adjusted for competing causes of death. A nonparametric method of estimating non-lethal disease incidence has also been investigated.

MAJOR FINDINGS: Nonparametric dose response curves have been developed which employ the area under truncated cumulative mortality curves adjusted for specific causes of death.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These methods are much more efficient in their use of experimental data than the classical interval techniques of mortality estimation which require much larger sample sizes. Also a clearer picture of mortality patterns is produced.

PROPOSED COURSE: A continuing effort will be made to develop procedures for interpreting data from survival studies. Specific attention will be given to (a) dose response including low-dose extrapolation, (b) incomplete cause of death information and (c) disease dependencies.

PUBLICATIONS

Hoel, D. G.: A representation of mortality data by competing risks. Biometrics 28, 475-488, 1972.

Hoel, D. G. and Walburg, H. E.: Statistical analysis of survival experiments. J. Nat. Cancer Inst. 49, 361-372, 1972.

Serial No.: NIEHS-B-008
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Molecular Orbital Calculations on Some Polychlorinated Biphenyls and Other Biphenyl Derivatives"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: M. W. Anderson, Ph.D.

OTHER INVESTIGATORS: N. K. Wilson, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is to determine the electronic distribution in some polychlorinated biphenyls and other biphenyl derivatives and to investigate the energy surface as a function of the dihedral angle between the two rings. This information will be used to help interpret the NMR spectra of these compounds and to investigate the conformational properties of these compounds.

METHODS EMPLOYED: The all valence electron CNDO/2 procedure in molecular orbital theory was used to perform the calculations. This is a semi-empirical procedure which has had success in predicting the correct trends in homologous series of compounds.

MAJOR FINDINGS: Calculations and experimental results indicate that the rings of the polychlorinated biphenyls which have chlorine attached to the 2,2',6 and 6' positions are very restricted in their rotational motion with respect to each other, i.e., the energy surface has a steep minimum with respect to the dihedral angle between the two rings. For substitutions in other ring positions, the energy is essentially independent of angle. Correlation between electronic charge distribution and proton and ^{13}C chemical shifts have been obtained for the chlorinated biphenyls which we have investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The energy surface studies are important because the ability of these biphenyls to bind to macromolecules or to undergo metabolism might depend upon the ease with which they can undergo conformational changes.

PROPOSED COURSE: The conformational properties will be further investigated by performing similar type calculations with another semi-empirial molecular orbital procedure, MINDO/2.

Serial No.: NIEHS-B-014
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Nonparametric Tests in Biometry"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: K. M. Patel, Ph.D. and D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	1.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To develop approximate power functions of nonparametric procedures for testing the equality of several treatments. (2) To develop a nonparametric sequential probability ratio test (based on ordered responses) for comparing two treatments.

MAJOR FINDINGS: (1) Assuming small differences in effects when the responses are governed by a known multidimensional density, the approximate power functions are developed. (2) Assuming Lehmann's alternatives, a sequential nonparametric test leading to an early decision is developed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: (1) Investigators are often interested in detecting the differences in effects with certain probabilities using nonparametric methods. The approximate power functions are useful in computation of these probabilities. (2) A clinical trial or survival study in which the effects of two treatments are compared may be designed so that the treatments are started simultaneously on all subjects under study. If the interval of time from the start of treatment to some clinical end point is the response, then data will become available as an ordered sequence. The sequential nonparametric test is useful in such studies in that the experiment may often be terminated early thus reducing costs.

PROPOSED COURSE: To study the performance of the sequential nonparametric test as compared to the Alling sequential nonparametric test on early decision.

PUBLICATIONS

Patel, K. M. and Hoel, D. G.: A nonparametric test of interaction in factorial experiments. J. Amer. Statist. Assoc. (in press).

Patel, K. M.: Hajek-Sidak approach to the asymptotic distribution on multivariate rank order statistics. J. Multivariate Analysis (in press).

Serial No.: NIEHS-B-017
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of Potential Time-Space Clustering of Neural Tube Defects in a Human Population"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: M. D. Hogan, Ph.D. and D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: Janice Carver

COOPERATING UNITS: North Carolina State Board of Health

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate fetal and infant deaths occurring in the state of North Carolina between 1947 and 1970, and to determine if there is any evidence of time-space clustering of deaths attributable to malformations of the central nervous system.

METHODS EMPLOYED: All possible pairs of cases of CNS deaths within various defined time-space grids are being examined for indications of clustering by use of Knox's test criteria and by Mantel's generalized regression approach. When a result attains significance, an attempt will also be made to estimate the exact permutational distribution of the number of pairs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because neural tube defects constitute one of the major causes of death among infants, and because many investigators feel that the onset of CNS anomalies may have a strong environmental component, any exploration of the etiology of this disease would have both bio-medical and Institute program relevance.

PROPOSED COURSE: (1) Fetal CNS deaths are being examined for time-space clustering using Knox's criteria. (2) When preliminary analyses indicate possible clustering, more detailed analyses of both fetal and infant deaths will be conducted based on Mantel's test procedure. (3) Auxillary tests for separate time and space effects will also be performed.

Serial No.: NIEHS-B-018
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of Mathematical Pharmacokinetic Models"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: M. W. Anderson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the various types of mathematical pharmacokinetic models and to determine the feasibility of using these models in a pharmacokinetic study of environmental contaminants.

METHODS EMPLOYED: The pharmacokinetic type model developed by R. L. Dedrick and D. S. Zaharbo for several drugs will be used to develop a pharmacokinetic model for environmental contaminants.

MAJOR FINDINGS: To study the pharmacokinetic properties of an environmental contaminant, the modeling approach developed by Dedrick et al has more desirable features than that taken in developing the classical compartmental type models. Since the Dedrick model is constructed from basic biochemical and physiological parameters such as enzyme kinetic parameters, tissue and plasma binding data, blood flow rates and organ sizes, more insight into the mechanisms governing the rates of absorption, distribution, metabolism and excretion of a chemical in intact animals can be obtained with this type model. By constructing this model for several species, extrapolation to man may be possible. If a successful model can be developed for a chemical under experimentally feasible exposure (single or multiple i.v. dosages), then predictions of the pharmacokinetic behavior may be possible under more natural exposure (chronic low-level exposure). The construction of a Dedrick et al type pharmacokinetic model requires more experimental data than that required to construct the compartmental type model. However, it is felt that by constructing this type model for

phototype compounds, a better understanding of the pharmacokinetic behavior of environmental contaminants can be obtained.

The mathematical form of this model is a system of simultaneous differential equations. A general purpose computer program for solving these equations has been obtained from Dedrick's group.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the rates of absorption, distribution, metabolism, and excretion of a chemical in intact animals is required in order to understand the toxicological effects of the chemicals.

PROPOSED COURSE: To determine if it is possible to construct a Dedrick et al type model for an environmental contaminant(s) which is(are) representative of a large class of environmental contaminants. The prototype compound(s) will have different chemical and physical properties than the drugs previously investigated by Dedrick et al. It will be more lipophilic and have a longer biological half life. These properties are typical of many environmental contaminants.

Serial No.: NIEHS-B-019
1. Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Mathematical Models for Estimating Mutation Rates in Cell Populations"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: D. G. Hoel, Ph.D. and K. S. Crump, Ph.D. (Louisiana Tech)

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To construct probabilistic models for describing the number of mutant cells in a cell colony as a function of time, and to develop estimates of cell mutation rates for the various models while taking into account experimental laboratory procedures.

METHODS EMPLOYED: Both Markovian and various types of deterministic growth were assumed for mutant and nonmutant cells. Mutants were assumed to be generated as a Poisson process. From these assumptions, the number of mutant and nonmutant cells may be expressed as functions of time.

MAJOR FINDINGS: Methods for estimating the cell mutation rate have been developed based on the models for cell growth. These estimates take into account laboratory procedures for periodic sampling of the cell cultures. Also the effects of phenotypic delay have been studied and methods for adjusting the mutation rate estimates have been developed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is necessary both to develop better and more realistic methods for estimating cell mutation rates and to understand the effects of the mathematical assumptions which are a basis for mutation rate estimation.

PROPOSED COURSE: To continue to improve mutation rate estimation and to develop estimates for the diploid locus case.

PUBLICATIONS

Hoel, D. G. and Crump, K. S.: Estimating the generation-time distribution of an age-dependent branching process. Biometrics (in press).

MUTAGENESIS BRANCH

MUTAGENESIS BRANCH
Summary Statement

The primary thrust of NIEHS' effort in mutagenesis is focused on the evaluation and definition of risks to both human germinal and somatic tissues. The Mutagenesis Branch, organized late in FY72 and anticipated to expand to full capacity within the next several years, has addressed itself to the problem by: (1) attempting to identify specific mutagenic hazards in man's environment, (2) developing new and comprehensive mutational assay systems having a high degree of relevance to human health, (3) developing a better understanding of the molecular basis of mutation in higher organisms and (4) attempting to understand the role of repair on the process of mutation-induction.

To complement and support these efforts, the Mutagenesis Branch undertook Collaborative Research studies at the University of Texas, Austin, whose activities include the development of techniques that can be applied to the problem of monitoring human populations for somatic mutations, and with the University of Wisconsin to develop an assay for determining spontaneous and induced rates of gene mutation on human somatic cells in culture. By Interagency Agreement we are supporting the refinement and evaluation of systems capable of detecting airborne chemical mutagens at the Brookhaven National Laboratory, and we are continuing to support the Environmental Mutagen Information Center at the Oak Ridge National Laboratory. This Center is a unique resource for the collection, storage and dissemination of information on the existence and activity of mutagens in the human environment. As part of our effort to identify specific biological hazards in our environment that may be mutagenic, a study has been started at the Institute for Medical Research to investigate and characterize virus-induced mutagenesis with special emphasis on those viruses found in vaccines. To obtain a better understanding of gene mutation at the molecular level, Miles Laboratory has been established for characterizing mutants induced by environmental chemical mutagens and carcinogens in our intramural research program. During FY73, efforts were made to promote expansion of programs in the Collaborative Research area which are intended to become an integral part of the Branch's research activities.

It is recognized that the nature of the problems in environmental mutagenesis is so broad and requires such diverse expertise in sophisticated technology as to far exceed what any single agency or institution could alone provide. It is, therefore, essential that program development in the Collaborative area be a continuing effort of highest priority. It is further anticipated that efforts initiated in FY73 will lead to the establishment of the following Collaborative Research programs for FY74: (1) the development of a new mouse strain to maximize the sensitivity of assays for gene mutations at specific loci in germ cells using electrophoretic variants and (2) the development of a new mouse strain carrying suitably marked inversions, which can be utilized for detecting recessive lethal mutations over a major portion of the genome. Both strains will provide a new assay for the detection of gene mutations, the latter strain has the advantage that it should provide an assay of a much greater portion of the genome than the first strain.

A new line of work in this Section involves attempts to develop an assay for the detection of gene mutations in somatic cells using both the glucose-6-phosphate dehydrogenase system and the fetal hemoglobin system. Such an assay would provide the capability for assessment of somatic mutation frequencies in individuals as well as for population monitoring.

Recruiting of personnel for a new section on Population Monitoring and Epidemiology has been successful and at the beginning of FY 1974, Dr. Charles Langley will join our staff to develop better methods to extrapolate data collected on experimental organisms to man. Another aspect of the work of this Section will be to develop better methods to monitor the population both to determine the impact on man of various types of genetic damage as well as to determine whether the load of genetic damage in the population is changing.

The work on mycotoxins of Dr. Ronald Pero (formerly of the Cell Biology Branch) has given particular attention to the genus Alternaria. A new compound, altenuisol, has been identified and characterized. Both this compound and altenuene, which was identified two years ago, are being given an intensive toxicological evaluation. Because this fungus is a widespread contaminant of many agricultural commodities, these and similar studies are expected to provide data for a better assessment of their economic importance as well as their long-term toxicological effects on the health of man. This group has also analyzed various commercial food sources to determine the levels of contamination with toxigenic fungi. Individual fungal isolates have been identified and a more detailed study of toxigenic species is planned to identify the active toxins.

The Environmental Mutagen Information Center has increased its data bank to approximately 7500 entries. Of these, 1834 will be published in the "1971 Survey of Literature Reporting Chemical Mutagenesis." During the past few months, an agent reference list was developed which will now be made for all papers in the EMIC data bank and for the current literature. In addition, a report has been prepared for the FDA on "The Mutagenicity and Teratogenicity of a Selected Number of Food Additives." The newest development is a request for Dr. H. Kissman of the National Library of Medicine to enter EMIC's data bank into the national network data base, Toxicon. This should make this unique data bank more generally available to users and may reduce the number of questions handled at present by EMIC personnel.

Toxicological evaluation of hycanthone (which is used to treat schistosomias in the tropics) and its structural analogs, is being continued as a high priority Branch activity. The problem is one of unique theoretical interest as well as one of practical importance. Hycanthone is unique as a chemical mutagen in that it can produce frameshift mutations in eukaryotes, and the mechanism of mutation-induction is of great interest. We intend to continue to synthesize and study the toxicological properties of structural analogs to determine to what extent desirable pharmacological properties can be maintained and undesirable toxicological properties eliminated. This study has involved the synthesis of new compounds by Parke-Davis and tests for mutagenicity within various sections of the Mutagenesis Branch. Teratogenicity tests are being performed in collaboration with the Animal Science and Technology Branch and antischistosomal activity in collaboration with Dr. E. Bueding at

Within the past year, staff members of the Mutagenesis Branch have participated in the formation of a new Panel on Environmental Mutagenesis and Carcinogenesis under the U.S.-Japan Cooperative Medical Science Program. A similar Panel on Mutagenesis has been started with the Russians as part of the US-USSR Agreement on Cooperation in the field of Environmental Protection. To foster interest and understanding of current problems in environmental mutagenesis, staff members of the Mutagenesis Branch have organized an Interagency Panel on Environmental Mutagenesis, organized a Workshop on Evaluation of Mutagenicity Data and Extrapolation to Man and another workshop on DNA Repair Systems in Fungi, served as a member of the Mammalian Genetics Advisory Committee for the National Center for Toxicological Research and collaborated with staff members of the National Cancer Institute to set up a study on the mutagenicity of chemical carcinogens. Staff members are in communication with staff members of the Food and Drug Administration and the Atomic Energy Commission to develop complementary programs of research and to avoid unnecessary duplication of effort. Staff members have also lectured to numerous audiences in industry and at universities on problems in environmental mutagenesis. An active role has also been taken in coordinating the toxicological evaluation of hycanthone (used to treat schistosomiasis in the tropics). Closer communication is maintained with other laboratories work on this problem in this country and abroad. The collaborative study not only includes hycanthone and leucanthone, but additional structural analogs. In addition to providing basic data on structure-function relations, the study may provide clues for the development of a drug with the desirable pharmacological properties but not the undesirable toxicological properties. The Branch has also served as a host for numerous distinguished scientists from this country, England, Italy, Germany, The Netherlands, Japan, Belgium and Russia.

Although the Mutagenesis Branch has been organized for only a brief period and a great deal of effort has gone into the remodeling of laboratory facilities, considerable progress has been made in its intramural research program.

In the Somatic Cell Genetics Section, a comprehensive study of repair events has been continued with mammalian cells in culture. Drs. Patterson and Clive have been studying repair replication after EMS treatment of mouse lymphoma cells, and Dr. Meneghini has persued a similar line of work in opossum lymphocytes. Both systems provide an assay for rapidly testing environmental chemicals for either stimulation or inhibition of repair replication. In the mouse lymphoma cells, the relationship between repair of EMS-induced alkylation of DNA and forward mutation rates at the thymidine kinase locus is being studied to determine the role of repair on observed mutation frequencies. In another line of work, Dr. Patterson has been developing procedures to determine whether environmental chemicals react with DNA and to characterize the reaction products. In his studies with EMS, measurement of EMS-induced alkylation makes it possible to correlate dosimetric relationships with observed mutation frequencies at the thymidine kinase locus. Further studies in this Section on the types of repair mechanisms in normal mammalian cells will make it possible to determine the role of the various repair processes on mutation-induction at specific loci.

Simple and comprehensive tests for gene mutations and other types of genetic damage are being developed in various microorganisms in the Microbial and

Plant Genetics Section. Using a strain of diploid yeast synthesized for us by Dr. F. K. Zimmermann (Brooklyn College), Dr. Majdi Shahin is developing a screening system for mitotic recombination and mitotic gene conversion. The assay system can be used *in vitro* as well as *in vivo* as an indicator organism in the host-mediated assay with mice. This approach will provide a test of the original chemical as well as its metabolites. Dr. Dan Straus is developing strains of *Salmonella* and *E. coli* which will detect gene duplication, recombination and gene conversion. These new strains will complement and extend the capability of the *Salmonella* strains developed by Dr. B. Ames (University of California at Berkeley) and Dr. P. Hartman (Johns Hopkins University) for screening for point mutations. In another line of work, Dr. F. J. de Serres is studying mutation-induction in repair-deficient strains of Neurospora. These studies have shown both quantitative and qualitative effects including a strain that is completely resistant to mutation-induction after UV treatment. Two strains in which mutation-induction is markedly enhanced are being studied further because they might provide a more sensitive assay for genetic activity of weak mutagens or chronic levels of exposure than standard wild-type strains.

The *ad-3* system of *Neurospora crassa* is being used by Drs. Ong and de Serres to determine the correlation between carcinogenic and mutagenic activity of chemical carcinogens. Samples of induced mutants are being characterized by genetic tests to determine whether mutagenic carcinogens produce a particular type of genetic alteration. These data should provide fundamental information that may provide an understanding of the mutagenic activity of this class of compounds. This work is being carried out in collaboration with staff members of the National Cancer Institute with an ultimate objective of providing short-term tests for mutagenic activity that can help to suggest carcinogenic activity in man.

In the newly formed Mammalian Genetics Section, Dr. William Sheridan is utilizing known strain differences in sensitivity to mutagens to develop a battery of test strains, ranging from highly sensitive to highly resistant. Testing environmental chemicals for genetic activity should better approximate the range of effects that might be expected in an extremely heterogeneous population such as man.

In another project, he will also attempt interaction experiments to get a better estimate of the sensitivity of stem cells to mutation-induction in male mice. Spermatogonial stem cells appear to be completely resistant to the induction of dominant lethal mutation and factors influencing this lack of responsiveness will be further explored.

In the Biochemical Genetics Section, Dr. Malling and his associates are developing a new mouse strain for the detection of gene mutations in mammalian germ cells. The assay will detect both electrophoretic mobility and inactive enzyme mutations at the same locus. Because electrophoretic variants are already known at many loci, a new strain is being developed where assays can be made for changes at over 20 loci. The present strain in which analyses can be made at 9 loci is being used by Dr. Valcovic to determine the frequency of each type of mutation after x-ray exposure of adult males.

Johns Hopkins University. This study will provide basic information with regard to structure-function relationships and may provide clues as to what type of structural modifications may be required to produce an analog to replace hycanthone for chemotherapy of schistosomiasis.

In conclusion, during the past year we have been successful in recruiting key personnel for our intramural program, have started cooperative programs with other Branches in the Institute, have coordinated program development of the Branch with similar programs in other Federal agencies and have established lines of communication with newly developing scientific organizations for environmental mutagenesis abroad. Along with the organization of workshops and conferences, these approaches will be used to stimulate research and development in this rapidly developing area of environmental health science.

Serial No.: NIEHS-MB-001
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 12, 1972 through June 30, 1973

PROJECT TITLE: "Mutagenicity and Mutagenic Specificity of Chemical Carcinogens in Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Tong-man Ong, Ph.D.

OTHER INVESTIGATOR: F. J. de Serres, Ph.D.

COOPERATING UNITS:

MAN YEARS

Total:	1.2
Professional:	1.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Although many chemicals have been shown to be carcinogenic in man and other mammals, the mechanism of cancer induction is not yet understood. Due to the stability of neoplastic state, however, the somatic mutation theory has remained as one of the most attractive hypotheses for the origin of neoplasia. If the induction of cancer by chemical agents results from somatic mutation, one might expect a correlation between carcinogenicity and mutagenicity or carcinogenicity and some type of genetic alteration caused by these agents. It is of interest then to determine whether there is a correlation between carcinogenicity in mammals and mutagenicity in Neurospora of some interesting chemical carcinogens, and whether these carcinogenic chemicals cause a particular type of genetic alteration.

Some chemical carcinogens, however, require enzymatic activation for the expression of their biological activity. An in vitro system for metabolic activation is being developed with liver microsomes. This system can be used for testing the mutagenicity of those chemicals where metabolic activation is required for their mutagenic and carcinogenic activities.

METHODS EMPLOYED: The ad-3 test system of Neurospora crassa developed by F. J. de Serres is used in this study. With this system one can isolate ad-3 mutants in N. crassa and determine the type of genetic alterations at the molecular level, such as base-pair substitutions or frameshift mutations as well as at the chromosomal level (chromosomal deletions). In general, the

methods could be described as follows: Conidia from the heterokaryotic vegetative cultures of N. crassa were harvested in saline, washed and treated with different chemical carcinogens. The treated conidia were inoculated into 10 liters of Westergarrd's medium with the appropriate supplements. After 7 days incubation in the dark at 30°C the frequency of purple ad-3 mutants were determined in each flask. The isolated ad-3 mutants will be made homokaryotic for the adenine requirement, and tested to determine the frequency of chromosomal deletion, leakiness, complementation, and nonpolarized complementation pattern by complementation, dikaryon and trikaryon tests.

The mutagenicity tests for the chemicals requiring metabolic activation for biological activity are carried out with mouse liver microsomes added to the treatment solution. The treatment conditions were similar to those used by H. V. Malling on Salmonella.

MAJOR FINDINGS: Our previous studies have shown that there is a positive correlation between the carcinogenicity in laboratory mammals and mutagenicity in N. crassa among the 14 chemical carcinogens tested. Genetic characterization of ad-3 mutants induced by these chemicals are still in process; however, we have found that mutation induction by two closely related chemical carcinogens, 1-phenyl-3,3-dimethyltriazene (PDMT) and 1-phenyl-mono-methyltriazene (PMMT), in N. crassa are due to different mechanisms. Metabolic conversion from PDMT to PMMT likely did not occur when conidia of N. crassa were treated with PDMT. The spectrum of genetic alterations cuased by PMMT in Neurospora is similar to those caused by other potent chemical carcinogens, aflatoxins and -propiolactone. The mechanism of mutation induction by PDMT in N. crassa is not clear; however, mutation induction by PMMT could result from alkylation of DNA by the carbonion ion produced by PMMT as the mechanism proposed for its carcinogenic activity.

The results of our preliminary studies showed that with a slight modification of the liver microsome test system used by Malling for testing the mutagenicity of dimethylnitrosamine (DMN), a potent carcinogen, for which enzymatic activation is required for the expression of its carcinogenicity, in Salmonella could be used for testing the mutagenicity of DMN in Neurospora. DMN is not mutagenic in N. crassa when conidia were treated. However, the ad-3 mutation frequency significantly increased over the spontaneous frequency after the conidia were treated with DMN and liver microsomes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity and mutagenic specificity of chemical carcinogens could provide information concerning the relationship between carcinogenesis and mutagenesis of chemical agents. Furthermore, the studies on the mutagenicity of chemical carcinogens in N. crassa might provide a system that could be used for screening carcinogenic chemical agents in the human environment.

PROPOSED COURSE: Future study will follow this program. More extensive studies on the mutagenicity and mutagenic specificity of chemical carcinogens, will be carried out. Much effort, however, will be devoted to those chemical carcinogens that metabolic activation is necessary for the expression of their carcinogenic activities.

Serial No.: NIEHS-MB-002
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Mutation-Induction in Repair-Deficient Strains of Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Frederick J. de Serres, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.6
Professional:	0.6
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Recent studies have shown that the process of gene mutation is under genetic control, and marked quantitative differences have been observed in mutant strains. In bacteria, repair-deficient strains have been found to act as mutators; and in yeast additional mutants have been observed to have the same properties. Mutant strains which show a higher level of response after mutagen treatment would be especially useful in screening programs to detect genetic activity resulting from low levels of exposure on weak mutagens. Exploratory experiments of our own have shown that repair-deficient haploid strains of Neurospora have marked quantitative and qualitative effects on the induction of point mutations at the ad-3A and ad-3B loci. The ad-3 test system is unique in that the mutations can be thoroughly characterized, including a presumptive identification of the genetic alterations in the point mutations at the molecular level. All possible effects were found including a strain which is completely resistant to mutation-induction after UV irradiation. In Neurospora, by using a two-component heterokaryon, it is possible to mimic a diploid organism and to induce, recover and analyze specific locus mutations resulting from both point mutation and (chromosome) deletion. By making these heterokaryons homozygous for various repair-deficient mutations, it is possible to determine their effect on both classes of specific locus mutations.

METHODS EMPLOYED: Crosses have been made of selected repair-deficient mutants (i.e., uvs-2, uvs-3, uvs-4 and uvs-6) to obtain suitably marked heterokaryons homozygous for each mutation. These four heterokaryons will then be used in forward mutation experiments to determine their effect on mutation-induction in the ad-3 region. These experiments will provide data on quantitative effects when data are compared with those obtained from a control, wild-type heterokaryon. Samples of ad-3 mutants will be collected in each experiment for characterization by a variety of genetic tests. Comparison of the spectrum of genetic alterations produced in each of these repair-deficient heterokaryons with the spectrum obtained in our standard wild-type strain will provide data on qualitative effects.

MAJOR FINDINGS: Heterokaryons homozygous for uvs-2 and uvs-4 have been constructed and exploratory forward-mutation experiments with UV are in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Neurospora has been used as an indicator organism in the host-mediated assay to assay for the induction of gene mutations as they occur in man. The present experiments with repair-deficient strains are expected to provide more sensitive strains for these experiments as well as providing basic information on the processes of gene mutation and the role of repair in the production of point mutations and chromosome detections. Are these two classes of mutations produced by qualitatively different genetic alterations which are subject to different processes of repair?

FUTURE COURSE: Future work with these strains will involve tests with a variety of specific chemical mutagens as well as UV and x-irradiation. Additional exploratory experiments will be started to determine the role of additional repair-deficient strains on mutation-induction. We need to develop strains which can be used to evaluate environmental chemicals under normal conditions of exposure; these are usually low levels over long periods of time.

Serial No.: NIEHS-MB-003
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Mutagenicity and Mutagenic Specificity of Environmental Mutagens in Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Tong-man Ong, Ph.D.

OTHER INVESTIGATORS: F. J. de Serres, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.6
Professional:	0.6
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Hycanthone and leucanthone, chemicals useful in the treatment of schistosomiasis, are attracting wide concern because of their potential carcinogenic and mutagenic hazard to treated individuals in the human population. It is of great interest to determine whether structural analogs of these agents are mutagenic in Neurospora and to determine the relationship between chemical structure and biological activities.

METHODS EMPLOYED: The ad-3 test system in a two-component heterokaryon of Neurospora crassa developed by F. J. de Serres is used in these studies as an assay for the production of gene mutations. With this system one can isolate ad-3 mutants resulting both from point mutation and deletion and determine the type of genetic alterations in the point mutations at the molecular level, (e.g., base-pair substitutions or frameshift mutations). In general, conidia from the heterokaryotic vegetative cultures of N. crassa were harvested in saline, washed and treated with different chemicals. The treated conidia were inoculated into 10 liters of Westergaard's medium with the appropriate supplements. After 7 days incubation in the dark at 30°C, the frequency of purple ad-3 mutants was determined. The isolated ad-3 mutants are made homokaryotic for the adenine requirement and characterized by a series of simple genetic tests.

MAJOR FINDINGS: The mutagenicity of hycanthone, a hycanthone analog (8-cholro-2-[2-diethylamino) ethyl]-2H-[1]-1-benzothiopyrano [4,3,2-cd]-indazole-5-methanol monomethane sulfonate), leucanthone and two leucanthone analogs

(8-chloro-2-[2-(diethylamino) ethyl]-5-methyl-2H[1]-benzothiopyrano [4,3,2-cd]-indazole monomethane sulfonate and 2-[1]-(diethylamine) ethyl]-5-methyl-2H-[1]-benzothiopyrano [4,3,2-cd]-indazole monomethane sulfonate) were tested in N. crassa. The results showed that all five compounds are mutagenic and are very toxic in Neurospora. The toxicity and mutagenicity of the analogs are greater than either hycanthone or leucanthone. The mutation frequencies observed varied from 0 to 1000-fold over the spontaneous frequency depending on the compound and concentrations used.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of hycanthone, leucanthone and their structural analogs are essential and important, because hycanthone is widely used for treatment of schistosomiasis in the human population. It is important to try to determine the potential mutagenic hazard of this compound to treated individuals. Studies on the relationship between structures and mutagenic activities of hycanthone and its analogs might lead to a production of a non-mutagenic compound that can be used for the treatment of schistosomiasis. This study will also provide basic information with regard to structure function relationships which will be important for all of our future work with mutagenic environmental chemicals. We need to know whether we can develop structural analogs of desirable but mutagenic chemicals that will retain desirable pharmacological properties but not undesirable toxicological properties.

PROPOSED COURSE: Future studies on the mutagenicity and mutagenic specificity of drugs and environmental mutagens will be focused on those environmental agents that are closely related to human health.

Serial No.: NIEHS-MB-004
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Detection of Chemical Mutagens with Salmonella typhimurium"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Daniel S. Straus, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Professor Bruce N. Ames
Department of Biochemistry
University of California
Berkeley, California 94720

Professor Philip E. Hartman
Department of Biology
Johns Hopkins University
Baltimore, Maryland 21218

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Ames, Hartman and coworkers have developed a highly sensitive set of Salmonella typhimurium strains for detecting base-pair substitution and frameshift mutations. They have also developed a set of strains which may be used to screen for agents which kill bacteria by reacting with DNA. The general objective of this project will be to use these strains to analyze the mutagenic activity of certain chemicals of environmental and pharmacological interest. One specific problem of medical interest has been approached thus far using the Salmonella tester strains. This has been an analysis of the mutagenic activity of a series of compounds which are structurally related to the drug hycanthone. The objective has been to find a drug which is not mutagenic but which is as effective as hycanthone in treating schistosomiasis.

METHODS EMPLOYED: (1) A reverse mutation test is employed in which four histidine-requiring mutants are reverted to prototrophy. One of these strains is specifically reverted by base pair substitution mutagens; the other three strains are reverted by frameshift mutagens of three different specificities. (2) A repair test is employed which detects chemicals which kill bacteria by

reacting with DNA. (3) Each of the above tests may be performed in the presence of liver microsome enzymes to determine whether mutagens are enzymically activated.

MAJOR FINDINGS: A number of thiozanthene derivatives which are structurally related to hycanthone have been tested for their ability to induce frameshift mutations. All of the compounds having a hydroxymethyl substitution in the 4 position of the thioxanthene ring system have been shown to be qualitatively similar to hycanthone in the type of frameshift mutations induced. Although quantitative differences in the mutagenic activity of various compounds in this series have been observed, no hydroxymethyl substituted thioxanthene derivative has been found which is active as an antischistosomal agent and not mutagenic.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The bacterial mutagen tester strains represent a rapid, simple, and highly sensitive system for screening for certain types of mutagenic activities. In combination with microsomal enzymes, they may be used to detect metabolic activation of various mutagens and carcinogens. The tester strains will, therefore, be useful in screening for the mutagenic activity of environmental chemicals.

PROPOSED COURSE: In collaboration with Ames and Hartman (cooperating units above) some new strains may be added to the bacterial mutagen tester series. In particular, it would be useful to have a sensitive forward mutation test which could be performed directly on solid medium. Numerous compounds of environmental and pharmacological interest may be studied. The results obtained with other organisms currently under study in the Mutagenesis Branch.

Serial No.: NIEHS-MB-005
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 thourgh June 30, 1973

PROJECT TITLE: "Development of a Screening System for Mitotic Recombination and Gene Conversion in Yeast in the Absence and Presence of Metabolic Activation"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: M. M. Shahin, Ph.D.

OTHER INVESTIGATORS: F. J. de Serres, Ph.D., H. V. Malling, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: A project has been initiated to develop a screening system for detection of specific changes of the genetic material other than mutations. The two processes are known as mitotic recombination (MR) and mitotic gene conversion (MGC). Mitotic recombination is a reciprocal process resulting from exchanges between homologous chromosomes. Mitotic gene conversion is a nonreciprocal event. Both genetic changes can lead to homozygosity of the recessive genes in diploid heterozygotes. Thus, these processes can conduct to the phenotypic expression of recessive genes. Mitotic recombination and gene conversion occur in diploid cells and has been shown to be induced with a variety of mutagenic and carcinogenic agents as well as ionizing radiations and ultraviolet light. Both are serious genetic changes, because their occurrence might lead to cancer or to other diseases in man. It should be noted, that there is no test system for the detection of these phenomenon in mammalian cell lines. Certain Saccharomyces cerevisiae diploid strains are convenient eucaryotic organisms in which mitotic recombination and mitotic gene conversion can be studied after treatment with chemical or physical agents and related to higher organisms.

METHODS EMPLOYED: At present, experiments are in progress to show that different and independent screening systems might be used for the detection of the potential chemical agents to induce mitotic recombination and mitotic gene conversion. The first approach will be the use of 3 specific S. cerevisiae

strains in vitro system and the characterization of the recently isolated D5 strain. The second system will be the use of these 3 strains of S. cerevisiae as indicator organisms in the host mediated assay with mice. This will provide a test of some of the metabolic breakdown products of the chemical resulting from mammalian metabolism. In the third system in vitro tests for metabolic activation will be developed using mouse and human liver microsomes. In all three systems hycanthone methanesulfonate (etrenol, a drug for treatment of schistosomiasis=bilharziasis) and some of its sturctural analogues will be tested. The two potent carcinogens, dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) which are considered to be nonmutagenic in microorganisms will also be included in our investigations. It is known that the breakdown products of these compounds in microsomal test system of liver, host-mediated assay and Udenfried's enzyme free hydroxylation system are the active mutagens.

MAJOR FINDINGS: Experiments are in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Physical, chemical and viral agents can cause normal cells to become cancerous. Certainly not all of these agents can cause cancer by the same mechanism. However, most carcinogens are capable of inducting mutations. Because mutation testing does not reveal all genetic damage that can be induced, therefore it is is necessary to test each agent suspected of being genetically active, for induction of MR and MGC, the other two possible changes of the genetic material. The present system might provide evidence that MR and MGC does occur in mammalian cell lines. The system can then be used as a tool for detection of potential agents to induce genetic damage.

PROPOSED COURSE: An effort will be made to develop a screening system for MR and MGC in yeast in the absence and presence of metabolic activation. In addition, some properties of D5 strain will be explored, and a comparison between the effects of hycanthone and its analogues will be made.

Serial No.: NIEHS-MB-006
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Detection of Gene Duplication, Reciprocal Recombination, and Gene Conversion in Salmonella typhimurium"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Daniel S. Straus, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.7
Professional:	0.7
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Although the induction by radiation and chemical mutagens of point mutations in bacteria has been extensively studied, relatively little is known about the induction in bacteria of genetic events involving the rearrangement of longer segments of DNA. Included in the latter class of genetic events are duplications, inversions, translocations, deletions, reciprocal recombinational events, and gene conversional events. The general objective of this project is to develop strains of Salmonella typhimurium and Escherichia coli which can be used to screen for some of these events and to quantitatively assess the effect of chemical mutagens and radiation on their frequency. In particular, bacterial strains are being developed to study gene duplication and recombinational events.

METHODS EMPLOYED: (1) Gene Duplication: It is known that certain mutants having nutritional requirements can revert to prototrophy by duplication of the mutant gene. In particular, a class of glycine-requiring mutants affected in the structural gene for the glycyl-tRNA synthetase (glyS) can revert to prototrophy by duplication of the glyS locus. The frequency with which this duplication occurs spontaneously and after treatment with mutagens may be quantitated. (2) Reciprocal recombination and gene conversion: Strains are being constructed which have non-complementing mutations in histidine biosynthesis on the chromosome and on an episome carrying the histidine operon. These strains require histidine and revert to prototrophy by recombination between the coromosome and episome. Reciprocal and non-reciprocal recombinational events can be distinguished by genetic analysis.

MAJOR FINDINGS: (1) Gene Duplication: Duplication of the glyS region has been found to occur at an extremely high spontaneous frequency ($>10^{-4}$) in E. coli. The spontaneous frequency is increased significantly (five to ten fold) by treatment with ultraviolet light. The frameshift mutagen ICR 372 also increases the rate of gene duplication. (2) Recombination: Strains carrying a number of histidine deletions on the histidine episome have been constructed. It has been shown that the configuration of the histidine operon on the episome is such that these strains revert only by the transfer of DNA from the chromosome to the episome. Ultraviolet light has been shown to increase the frequency of this recombinational event.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A simple, highly sensitive set of bacterial tester strains has been developed by Ames and coworkers for detecting point mutations in Salmonella typhimurium. This project is concerned with developing tester strains for screening genetic alterations other than point mutations.

Gene duplication and genetic recombination are biological processes of fundamental importance. Both processes have been postulated to play an important role in evolution. Because of the drastic effect which duplication and recombination can have on cellular phenotype, it is of interest to determine whether environmental chemical agents to which humans are exposed influence the frequency with which these two types of events occur.

PROPOSED COURSE: It is not at present known whether the high spontaneous frequency of duplications in the glyS region is an anomally of that region or whether duplications occur at a high frequency in all regions of the chromosome. To answer this question, it will be necessary to find mutants in other regions of the chromosome which revert by duplication. The observation that both UV and ICR 372 stimulation duplication is of interest because these two agents induce completely different types of damage to DNA. A systematic screening of mutagens having other modes of action will be undertaken to determine which other agents affect frequencies of duplication. The long range goal will be to understand on a molecular level how gene duplication occurs and how this process is affected by mutagenic agents.

Similar studies will be undertaken to find which mutagens affect the frequency of recombination. It will be of interest to compare the results obtained with the results of experiments on gene conversion and reciprocal recombination in yeast.

Serial No.: NIEHS-MB-007
1. Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Electrophoretic Mobility Mutation Analysis in Mammals"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Lawrence R. Valcovic, Ph.D.

OTHER INVESTIGATOR: Heinrich V. Malling, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	2.1
Professional:	1.4
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: Classically, the detection of gene mutations in mammalian germ cells has relied upon the induction of visible recessive mutations. These studies have not yet identified the molecular event responsible for producing the mutant phenotype. However, the study of specific enzyme mutations using electrophoresis has the capability of detecting mutations that result in a non-functional protein or a functional protein with altered electrical charge. Our objective is to develop a single mammalian test system that will detect both electrophoretic mobility (EPM) and inactive enzyme (IAE) mutations in the same loci. This will enable us to determine the relative frequencies of these two types of mutational events in response to various types of mutagenic agents.

METHODS EMPLOYED: Direct approximate estimation of the ratio between mutations resulting in inactive enzyme (IAE) mutations and EPM-mutations can be estimated from the changes in the zymogram of heterozygous offspring from a cross between two inbred strains which differ in the electrophoretic patterns of several enzymes. In the simplest case let us assume the normal heterozygote will have 3 bands for a particular enzyme. A mutant induced in either parent can result in one of three different electrophoretic patterns: (a) the presence of any one band indicating that the mutant is inactive; (b) the presence of any two bands, one at the normal position for one of the parents, and the other at any position indicating that the inactive enzyme will form an active aggregate with the wild-type enzyme; and (c) the presence of three bands, two with changed positions, indicating an EPM-mutation. Because case (a) and (b)

indicate inactive enzymes and (c) an EPM-enzyme, a direct estimation of the ratio between IAE-mutations and EPM-mutations can be made.

We have sleected two inbred mouse strains which differ with respect to nine enzyme loci. Additional non-variant bands will be apparent with some of the strains; and while the number of loci that these represent are unknown, EPM mutations may be detected at these positions.

Because it is important to correlate this system with Russell's specific locus test in mice, the first mutagen will be ionizing radiation. Adult males will be irradiated and after the sterile period will be mated to non-irradiated females. Electrophoresis will be performed on homogenates of tissues obtained surgically from F_1 adults.

MAJOR FINDINGS: Our main effort in this period has been directed toward establishing technique protocols for tissue preparation and electrophoresis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of chemicals may increase the genetic load of the human population which may possibly present a health hazard. There are two approaches that may be considered to avoid such an event. First, use animal test systems to detect chemicals which are mutagenic and prohibit or limit their use. Second, use mutant alleles at selected loci in the human population and monitor these for several years to determine whether there is an increase in the frequency of these alleles. The system under development will be pertinent to both of these approaches. It will survey a large enough number of different loci to minimize the cost of testing chemicals for potential mutagenicity and because many of the genes in the test are comparable to genes in the human population, it will also provide a baseline for the study of the mutation rate of electrophoretic mobility mutations in human population. Furthermore, because most of the loci code for specific enzymes, purification and biochemical analyses can be employed to determine the nature of the mutational event, i.e., base-pair substitution and/or addition/deletion in the DNA.

PROPOSED COURSE: The primary effort in the future will be the eldctrophoretic screening to detect radiation induced mutations. Tissue from 3,000 offspring from an inter-strain cross (male parent irradiated) will be analyzed for nine different enzyme systems. All new mutations will be characterized genetically. Also, enzyme purification procedures will be established to enable biochemical studies of induced mutations. The search for new enzyme polymorphisms will be initiated.

Serial No.: NIEHS-MB-008
1. Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Use of Mammalian Somatic Cells in the Detection of Mutational Events"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. V. Malling, Ph.D.

OTHER INVESTIGATORS: L. D. Claxton

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this investigation is to develop methods by which the frequency of somatic mutation at several genetic loci in experimental animals can be measured. Because blood is a readily available source of somatic cells that have a high turnover rate, we will initially attempt our studies with the use of blood. However, other tissues will be examined at a later date by a variety of techniques. Although we will attempt to explore a glucose-6-phosphate dehydrogenase system and a fetal hemoglobin system, a thorough literature search will be attempted in order to find other possible systems. The detection of mammalian somatic mutation would provide advantages in mutation research for the following reasons: (1) human cell samples could possibly be used for the study of mutational rates in man, (2) in mutagenic test systems, the animals own cells would be used, which would avoid the immunological reactions occuring in the host-mediated assay, (3) chronic studies of compounds in low doses could be initiated and (4) fewer animals and smaller facilities could be maintained for the study of mammalian mutagenesis.

METHODS EMPLOYED: Glucose-6-phosphate dehydrogenase is an enzyme that occurs in variety of inherited forms in various mammals. These rare variants can be recognized by staining techniques and electrophoresis. By using the staining technique and varying one of the parameters (i.e., its coenzyme), one can look for individual variant cells. When the normal coenzyme is used, approximately 95-98% of the lymphocytes are stained. However, with a change in coenzyme less than one per 100,000 of the cells stain. Fetal hemoglobin is not

normally found in adult erythrocytes; however, careful staining with an established technique does demonstrate a few positively stained cells in the adult animal. Only a preliminary attempt has been made using either of these methods, and present efforts are aimed at preventing the false positives caused by common fixing agents.

MAJOR FINDINGS: Although work in this area is in its infance, we feel that early results demonstrate that a somatic mutation system in mammals in feasible. We know, for instance, that genetic variants in the presently tried systems do exist. It has also been demonstrated that one can with histological techniques locate the rare stained cell or cells that indicate a mutational event.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Simply stated, this new mutation system would allow the determination of increased mutational events within an individual animal. The system could possibly be applied directly to human studies. Common disadvantages found in present mammalian systems (such as, very large numbers or animals, immunological interference, lack of chronic studies) could be overcome.

PROPOSED COURSE: Future efforst will try to determine the actual feasibility of these two systems and will also try to create other possible systems.

Serial No.: NIEHS-MB-009
1. Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Electrophoretic Characterization of Serum Pre-Albumins in Mus musculus"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. V. Malling, Ph.D.

OTHER INVESTIGATORS: L. D. Claxton

COOPERATING UNITS: None

MAN YEARS

Total:	0.9
Professional:	0.2
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: In order to develop a specific locus mutation system employing the use of electrophoretic variants, one must first determine which heterozygous alleles can be monitored by electrophoresis. Next, the investigator must determine the electrophoretic differences between his experimental and parent strains of animals. It has been our purpose to determine a relatively large number of heterozygotic alleles for mutation study. The discovery of serum pre-albumins in Mus musculus has added to the number of alleles; therefore, it has also been our purpose to do preliminary studies of the genetics and electrophoretic characteristics of these proteins.

METHODS EMPLOYED: Serum protein differences in different strains of a particular species can easily be determined by electrophoresis. Wanting to create the highest possible resolution in determining protein differences, we decided to use discontinuous polyacrylamine electrophoresis. Using 10 animals from each strain, nine inbred strains of Mus musculus were electrophoretically surveyed with the use of a general protein strain. According to recent literature, two strains, DBA/2 and C57BL/10, are genetically very different. These two were crossed to create an F_1 generation, and this F_1 generation was then backcrossed to each parent strain. Approximately fifty animals in the parents strains and one hundred animals from each cross were also studied electrophoretically.

MAJOR FINDINGS: Our electrophoretic technique clearly demonstrated a series of proteins called pre-albumins that have not been reported in the literature. Strain differences in these pre-albumins were also demonstrated; and in the

breeding portion of the experiment, DBA and C57BL/10 clearly showed banding differences in the pre-albumin area. The F_1 generation showed a combination of the two banding patterns. Although we have not finished the electrophoresis of the backcross serum samples, the banding appears to have linkage with the brown color genes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: New knowledge concerning electrophoretic technique and the genetic make-up of a common laboratory animal has been added to this area of basic science. We have also added to the number of heterozygotic alleles that are available for mutation study, and demonstrated the means by which more can be found.

PROPOSED COURSE: This basic research should be completed during this fiscal year at which time its results may be reported in the literature. This information may also be used in future projects for the Institute.

Serial No.: NIEHS-MB-010
1. Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Dimethylnitrosamine to a Mutagen by Mouse Liver Microsomes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: C. N. Frantz, M.D., H.V. Malling, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	.3
Professional:	.3
Other:	.0

PROJECT DESCRIPTION

OBJECTIVES: Some chemicals may be harmless to an organism until that organism metabolizes the chemical to a reactive product. Dimethylnitrosamine (DMN) is such a chemical. When metabolized by mammals, mainly in the liver, it becomes a toxic, carcinogenic, and mutagenic agent. Using this chemical, a rough assay to detect mutagenic compounds has been developed by one of the authors (H. V. Malling) in another laboratory. This assay employs mouse liver to metabolize the chemical; bacteria is the organism in which mutations may be detected. It is our purpose to refine the above system in terms of variations in metabolic activity of the mouse livers, i.e., variations due to concentration, strain difference, and treatment with enzyme inducers. Also, attempts will be made to elucidate the biochemical pathway which metabolized DMN to a mutagen.

METHODS EMPLOYED: The findings to date have been obtained using supernatant of mouse liver homogenate incubated with DMN and sufficient co-factors in the presence of S. typhimurium G-46, increasing the nu er of bacteria reverting from histidine dependence to histidine independence. Further work will involve the same system using a purified microsomal fraction from mouse liver. Simultaneous assays of the microsomes for the production of formaldehyde during demethylation of DMN will be performed. Demethylation is thought to be the reaction which metabolized DMN to a potent mutagen.

MAJOR FINDINGS: In refining the assay system, it was discovered that enzyme concentration is critical to the yield of mutations, and therefore to the potential usefulness and reliability of the assay. Above a critical enzyme

concentration, mutation yield becomes linear in relation to amount of enzyme. Therefore it can readily be compared to other biological events. Studies in the scientific literature have described a correlation between incidence of liver tumors from DMN and level of DMN demethylating activity in rats. We found that two agents known to decrease DMN demethylase activity in male rats, 3-methylcholanthrene and phenobarbital, increase the ability of the livers of male mice to metabolize DMN to a mutagen. Secondly, a marked variation was found between strains of mice in ability to metabolize DMN to a mutagen, but the same variation was not found for diethylnitrosamine (DEN).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The liver microsomal assay system for mutagenic compounds is one of many assay systems being developed and evaluated for screening of potential mutagenic compounds. The effect of enzyme level is necessary to the refinement of this assay system. The findings of differences between the carcinogenic effect of DMN in rat and the mutagenic effect of DMN metabolized by the mouse has yet to be explained. Possibilities include species difference in enzyme characteristics, a difference between carcinogenesis and mutagenesis in active agent or mechanism, or a lack of correlation of demethylation of DMN and production of a mutagenic compound.

PROPOSED COURSE: The next step must be correlation of enzyme activity and DMN mutagenic activity with respect to both strain differences in mice and enzyme induction with various drugs. These studies will hopefully elucidate the role of the demethylase in the formation of mutagenic compounds from DMN.

Serial No.: NIEHS-MB-011
1. Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Mouse Lymphoma Cell Host-Mediated Assay for Mutagens"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: C. N. Frantz, M.D., H. V. Malling, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.9
Professional:	0.9
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Man is exposed daily to a host of chemicals and drugs. There is at present no good way to determine the genetic effect directly on man of any of these agents but there are means of detection of genetic effects in small rodents and, more easily, in even smaller organisms. Bacteria and fungi are the easiest, cheapest organism to test for genetic damage, and animal cells in cell culture, somewhat closer to human cells in genetic composition, can also be handled in sufficient quantity to study rare mutational events. During the mammalian metabolism of certain drugs, highly mutagenic breakdown products are formed. A drug tested *in vitro* for mutagenicity without encountering mammalian metabolism may not show any mutagenic effect. In the host-mediated assay the indicator organism is injected into the mammal and kept there during the treatment of the animal with the drug. In this way, the indicator organism is exposed to the original drug and its metabolites. In most host-mediated assay microorganisms are used. The immunological reactions of the host to the microorganism may greatly influence the mutation data. In order to minimize the anitgen difference we propose to develop a mouse cell-mouse host-mediated assay. The L5178Y mouse lymphoma cell is placed intraperitoneally in the mouse strain of its origin, DBA/2J. This system would then be used to assay the *in vivo* mutagenic activity of various drugs and chemicals.

METHODS EMPLOYED: Thymidine kinase (TK), an enzyme normally found in all mammalian cells, phosphorylates thymidine to thymidine monophosphate. 5-Bromodeoxyuridine (BUdR) is an analog of thymidine which can also be phosphorylated by TK, but upon incorporation into DNA kills the cell. Thus

a mutation leading to markedly decreased or absent thymidine kinase activity can be detected by cell survival in the presence of BUdR. Cells lacking TK use an alternate pathway to form thymidine monophosphate. Continuous folate metabolism is required in this pathway, and when folate metabolism is blocked, TK-deficient cells die. In this manner TK-competent cells may be selected. This genetic locus in the L5178Y cell line was originally isolated and investigated by D. Clive and W. G. Flamm of NIEHS. The L5178Y mouse lymphoma cell line has been growing in vitro for many years. As is not unusual, the cells no not readily grow in vivo (intraperitoneally) but do grow readily after repeated intraperitoneal passage. This process of "adapting" the cells to in vivo growth may take many week-long passages in the host-mouse peritoneum. The cells must be growing actively when exposed to a test mutagenic agent in order to get consistent results. Also, one should have an approximation of the number of cells being treated and have sufficient cells, when the animal is sacrificed, to test for mutations. When the system is developed, a variety of known and possible mutagenic agents will be assayed. Testing a variety of known mutagens of different mechanism of action will define the versatility and sensitivity of the assay system.

MAJOR FINDINGS: The original TK-deficient and competent cell lines and the derivative cell line partially adapted to intraperitoneal growth were lost due to contamination of cultures by mycoplasma. A new mutant testing line was isolated, but with a very high spontaneous mutation rate. A great deal of sensitivity of the test system is therefore lost: e.g., if a given mutagenic treatment caused 10 new mutants per 10^6 cells, it is a significant difference (a two-fold increase) from a culture starting with only 10 mutants per 10^6 cells. However, if the original culture has 100 mutants per 10^6 cells, a 10% increase would not be statistically significant. An extensive search by other investigators (D. Clive) has so far failed to isolate another mutant test system with a low spontaneous mutation rate. The second mutant test system (with a high spontaneous mutation rate) has been adapted to intraperitoneal growth in DBA/2J mice in 9 week-long passages. If 10^6 adapted cells are injected i.p., about 30% are immediately recoverable. Over the first 3 days of their intraperitoneal stay, the number of recoverable viable cells drops to between 5 and 10% of the injected number. From that point the cells grow with a doubling time of about 1.5 days as compared to 0.5 days in vitro. By the 7th day there are sufficient cells to detect changes in mutant frequency, and by the 9th day the animals must be sacrificed to prevent intraperitoneal bleeding, which makes testing somewhat more difficult. This growth pattern is adequate for the assay system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At present, the only means of testing drugs and chemicals for ability to induce point mutations in mammals is the specific locus test, which requires a great number of mice. The host-mediated assay with lymphoma cells is a more convenient test system which may be used for extrapolation to possible results of human exposure to the agents tested. Furthermore, the thymidine kinase locus is a forward mutation system, thus allowing detection of all types of point mutations and small chromosomal deletions. Although it cannot detect chromosome rearrangements, additions or substractions, it will detect a wide variety of viable genetic effects which can be extrapolated to human cancer and genetic disease.

PROPOSED COURSE: In order to isolate from the adapted cell line a new cell line with a low spontaneous mutation rate, revertants from TK-deficient cells to TK-competence have been isolated and are being tested to determine the spontaneous mutation rate. Also, attempts will be made to isolate a new mutant tester strain from a different L5178Y cell line, which would then have to be adapted to *in vivo* growth. Once the appropriate cell line is obtained, the intraperitoneal assay system will be defined using mutagens which act by various mechanisms. The cell line will be explored for intravenous use in order to obtain a different type of *in vivo* exposure to mutagens. The cells would be recovered from the liver, where they are exposed to highest concentrations of those chemicals metabolized mainly in the liver. Another type of assay system using an *in vitro* liver preparation to metabolize potential mutagens and simultaneously expose the cells to the metabolic products is also being explored.

Serial No.: NIEHS-MB-012
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of Thymidine Kinase Enzymes in Independently Derived Mutant Lines of L5178Y Mouse Lymphoma Cells"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Donald Clive, Ph.D.

OTHER INVESTIGATORS: Peter Voytek, Ph.D., M. R. Machesko, Merritt D. Long

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: A number of genetic test systems have been developed, utilizing a spectrum of organisms ranging from prokaryotes to whole mammals. None is perfect for assessing mutagenic hazards to man: those organisms which best combine sensitivity and analytical capability are phylogenetically most distant from man, while, conversely, those species closest to man combine the disadvantages of long generation times, large size and great expense to their use. A compromise between those two extremes is afforded us by the use of cultured mammalian cells, which combine large numbers, relatively short generation times, ease of handling and low cost. However, such systems still lack the level of biochemical and genetic comprehension which elevates a mutagen assay system to a powerful genetic tool. Such understanding must develop as a wedding of genetic and biochemical studies on a number of mutant lines.

Genetically, the thymidine kinase (TK) locus of L5178Y mouse lymphoma cells can be mutated by a variety of physical and chemical agents. TK-deficient mutants can be isolated and later reverted to yield TK-competent mutants. (See Project Nos. NIEHS-M-004 and NIEHS-M-006). Thymidine kinase activities on the cell lysates confirm these designations. What is not known -- and what this project hopes to elucidate -- is whether a control gene is being mutated to shut off production of thymidine kinase, or whether the thymidine kinase structural gene itself is being affected. In the first case, analyses of all TK-competent lines should show no biochemical variations among the various thymidine kinase enzyme sources. On the other hand, the combination of a

forward and a reverse mutation in the structural gene need not yield precisely the same enzyme in the various revertani lines. And, even though enzyme activities are similar among these revertant lines (an expected result, because the very existence of the mutant is predicated on its possessing good thymidine kinase activity) other characteristics of the enzyme might differ widely (e.g., electrophoretic mobility, alterations in allosteric repsonses to activators and inhibitors, kinetic parameters).

METHODS EMPLOYED: For the production and isolation of the various mutant stocks see Project Nos. NIEHS-M-004 and NIEHS-M-006. Because the TK locus was diploid ($TK^{+/+}$) in the original lymphoma line, this cell line will be examined first, to assess the characteristics of these two (possibly identical) gene products. Several $TK^{-/-}$ mutants have been isolated from these $TK^{+/+}$ cells and characterized for enzyme activities and back-mutation (to $TK^{+/-}$) rates. A number of such $TK^{+/-}$ revertants will be utilized for TK enzyme characterization and ultimate comparison with the initial $TK^{+/+}$ enzyme(s) (See Project No. NIEHS____ for detailed biochemical methodology.

MAJOR FINDINGS: See Project No. NIEHS ____ for the biochemical findings. Further genetic analysis must wait until this methodology has been developed to the point where discretion among mutants is possible.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Complete understanding of how a particular mutagen acts depends ultimately on the level of sophistication of the genetic test system being used. This project aims at increasing our knowledge of the already proven TK heterozygote L5178Y mouse lymphoma mutagen assay system.

PROPOSED COURSE: Purification and characterization of the thymidine kinase enzymes derived from various $TK^{+/-}$ mutants will be continued.

Serial No.: NIEHS-MB-013
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Mutational Assay Systems Utilizing Mammalian Cells"

PREVIOUS SERIAL NUMBER: NIEHS-M-004

PRINCIPAL INVESTIGATOR: D. Clive , Ph.D.

OTHER INVESTIGATORS: M. R. Machesko

COOPERATING UNITS: None

MAN YEARS

Total:	0.50
Professional:	0.25
Other:	0.25

PROJECT DESCRIPTION

OBJECTIVES: Because of their large numbers, relatively short generation times, biological relevance to mammalian genetics and ease of handling, mammalian cells grown in suspension culture offer a highly sensitive system for assaying the mutagenicity of environmental agents. Elucidation of the mechanism by which such mutagenic agents exert their effects in mammalian cells cannot be accomplished by the sole use of the simple forward mutation assay system which we have developed. What is required is a combination of mutagenic and biochemical studies (see Project No. NIEHS-M-____), and for the development of mutant strains possessing high specificity in their responses to various mutagen classes. It is the objective of this project to develop mutant stocks and assess various genetic loci for their utility in effecting these aims.

METHODS EMPLOYED: Thymidine kinase- (TK-) deficient mutants of L5178Y mouse lymphoma cells are isolated as resistant colonies growing in soft agar cloning medium supplemented with 50 µg/ml of 5-bromodeoxyuridine (BUdR); this medium selects against TK-competent cells by virtue of their ability to effect a TK-mediated phosphorylation of BUdR which is ultimately lethal. TK-deficient cells are selected against by a medium supplemented with thymidine (T), hypoxanthine (H), methotrexate (M, to inhibit folic acid metabolism) and glycine (G) (THMG medium), whereas TK-competent cells can utilize the exogenous thymidine to overcome the methotrexate block and, hence, survive. Spontaneous mutation rates (forward and reverse) are determined by the rate of mutant accumulation in a culture and, where practicable, a fluctuation test. TK mutant phenotypes are confirmed by determining the rate at which their cell

lysates can phosphorylate thymidine (TK assay). The efficiency of mutant recovery in each of the selection media is determined in reconstruction experiments, utilizing standard mutant stocks. Potential chemical mutagens are assayed as follows: test cells of known genotype are treated with the compound for 2 h, the cells are washed, allowed to express genetic lesions for 48 h, cloned in selective cloning medium and scored for surviving mutant colony frequency after 7-10 days incubation.

MAJOR FINDINGS: The thymidine kinase (TK) locus in L5178Y mouse lymphoma cells appears diploid and the 3 possible mutant forms -- the $TK^{+/+}$ and $TK^{-/-}$ homozygotes and the $TK^{+/-}$ heterozygote -- have been isolated and characterized as a prerequisite to their use in this mutational assay system. The TK-competent clonal lines ($TK^{+/+}$ and $TK^{+/-}$) clone efficiently in soft agar cloning medium supplemented with THMG but are quantitatively killed when cloned in the presence of BUdR. On the other hand, TK-deficient ($TK^{-/-}$) cells are killed by THMG but are unaffected by BUdR. TK activities for the $TK^{-/-}$, $TK^{+/+}$ and 9 independently arising $TK^{+/-}$ back mutants are, respectively, 0, 61 and 27.5 $\pm$ 1.5 μmoles of thymidine phosphorylated/10^6 cells/minute of incubation. The $TK^{+/+} \rightarrow TK^{-/-}$, $TK^{-/-} \rightarrow TK^{+/-}$ and $TK^{+/-} \rightarrow TK^{-/-}$ spontaneous mutation rates are, respectively $\sim 5 \times 10^{-11}$ (based on recovery of only 1 mutant), 6.4×10^{-9} and 1.2×10^{-7} mutations/locus/generation. Reconstruction experiments have shown the recovery of nearly 100% of added $TK^{-/-}$ mutant cells when up to 8×10^4 $TK^{+/+}$ cells are plated per ml of BUdR-supplemented cloning medium; similar recoveries are obtained by co-plating $TK^{+/+}$ cells in the presence of up to 1×10^5 $TK^{-/-}$ cells per ml of THMG-supplemented cloning medium. The forward ($TK^{+/-} \rightarrow TK^{-/-}$) mutation rate has been greatly stimulated using known mutagens (X-irradiation; ethyl methanesulfonate) and was instrumental in demonstrating the mutagenicity of the drug hycanthone methanesulfonate, thus demonstrating the efficacy of this system in mutagen detection. Other genetic loci have been investigated for possible use in an extended mutational assay system. However, the mutation rate at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus was puzzlingly little stimulated over the spontaneous rate by both ethyl methanesulfonate and hycanthone methanesulfonate. Mehtotrexate-resistance (due possibly to transport defect or altered dihydrofolate reductase activity) showed some promise in its response to these two mutagens but requires a ten-fold increase in material over the TK system. Finally the adenine phosphoribosyl transferase (APRT) locus proved to be diploid, and will have to be made hemizygous in a usable $TK^{+/-}$ line before it can be further evaluated. Biochemical assays are performed on all mutant stocks amenable to such analysis. Attempts to develop $TK^{-/-}$ tester strains for use in a back-mutation assay (akin to that developed in Salmonella) have been thwarted by the necessity to induce defects of identical nature into both TK genes, leading to logistics which this lab has been unable to manage until just recently. A preliminary effort (unfruitful) has been made to alleviate this logistic problem by tailoring the TK locus to but a single revertible locus, the (for our purposed) "absent" locus being involved in an X-ray-induced chromosomal deletions. Such attempts though of taxing magnitude, have the advantage of being completed once a single such mutant is found; this hemizygous TK^{+} mutant can then be used to produce with great efficiency all of the mutagen class-specific tester strains desired.

The first $TK^{+/-}$ heterozygote which we isolated -- and with which we pursued our early studies on spontaneous and induced mutation rates -- was lost in a near lab-wide PPLO infection of unknown origin. Since then several independent $TK^{+/-}$ lines have been isolated using various means in an attempt to acquire a heterozygote possessing all of the favorable characteristics which made the TK system initially so ideal. The most important of these characteristics -- and the one which has proven most elusive -- is a low spontaneous forward mutation rate (1×10^{-7} mutations/locus/generation as opposed to the current range of 1×10^{-6} to 1×10^{-5}). These two classes of heterozygotes differ quantitatively in their mutagenic response to hycanthone methanesulfonate (the spontaneously hypermutable lines being ten-fold more mutable by hycanthone) but not in their response to ethyl methanesulfonate. We are currently pursuing our mutagen screening and analysis program (see Project No. NIEHS-M-005) utilizing one of these hypermutable heterozygotes; although such lines are less adequate for the study of weak mutagens than was the first heterozygote, they perform satisfactorily with the compounds presently under scrutiny. However, intense efforts in the isolation of more sensitive TK mutants are still in progress, being based on the known mutagenic differences listed above.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is integrated with the development of a mouse-mouse host-mediated assay for chemical mutagens (Project No. NIEHS-M-005) and with studies of the mutagenicity of environmental agents (Project No. NIEHS-M-006) and shares their significance. In conjunction, the three projects aim to (1) screen environmental compounds for mutagenicity; (2) distinguish between mutagenicity of a parent compound and of its metabolites; and (3) characterize mechanisms by which such mutagenic effects are produced.

PROPOSED COURSE: The detection of widely circulated weak mutagens determines to a great extent our continued quest for a $TK^{+/-}$ line which combines low spontaneous mutability with high sensitivity to weak mutagenic treatments. Having once isolated such a heterozygote from a rarely occurring $TK^{-/-}$ mutant, we feel that a repetition of this feat is possible; improving our chances for accomplishing this aim will hopefully be a consequence of understanding better the control of mutation induction. To this aim we are beginning to pursue studies on the capabilities these cells possess in the repair of genetic damage or various sorts, because successful repair is the most obvious means of maintaining a low spontaneous mutation rate.

A more exhaustive attempt to introducing hemizygosity at the TK locus will be initiated, using, perhaps, chemical mutagens of known mechanism of aciton to avoid large, possible dominantly lethal, chromosome deletions which might be associated with the excision of a TK gene.

Finally, depending on the success of acquiring a TK hemizygote, attempts will be made to construct specific TK^{-} tester strains to be used in a reverse mutation assay a' la Salmonella.

PUBLICATIONS

Clive, D., Flamm, W. G., Machesko, M.R., and Bernheim, N. J.: A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Res., 16:77-86, 1972.

Clive, D., Flamm, W. G., and Patterson, J. B.: Specific locus mutational assay systems for mouse lymphoma cells. In Hollaender, A. (Ed.): Environmental Chemical Mutagens. v.3, Plenum, New York-London, (In Press).

Serial No.: NIEHS-MB-014
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Safety Testing and Analysis of Environmental Compounds for Mutagenic Effect"

PREVIOUS SERIAL NUMBER: NIEHS-M-006

PRINCIPAL INVESTIGATOR: D. Clive, Ph.D.

OTHER INVESTIGATORS: M. R. Machesko

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The rapid increase in both variety and quantity of environmental mutagens is intimately associated with attempts to "better living through chemistry". It is presently possible to partake of these compounds via ingestion, inhalation and injection (e.g., nitrites and certain other food additives; TMP in automotive exhausts; and certain chemotherapeutic agents). By identifying specific compounds as mutagens it is hoped that a more cautious approach to their use will minimize the increased frequency of serious genetic disorders that exposure to such agents is expected to produce in subsequent generations.

We have developed an *in vitro* mutagen assay system (see Project No. NIEHS-M-004) utilizing thymidine kinase heterozygote ($TK^{+/-}$) L5178Y mouse lymphoma cells, which respond quite sensitively to treatment with known mutagens. Two classes of chemical mutagens (alkylating agents and hycanthone analogs) are currently being assayed in this system for the purpose of determining structure-activity relationships. The two classes of compounds represent mutagens of two distinct mechanisms of action (i.e., covalent alkylations of the DNA resulting primarily in base-pair alterations; and non-covalent intercalation into the DNA leading to spatial distenstions of the base-pair sequences and ultimate frame-shift mutations).

METHODS EMPLOYED: These are described in detail under Project No. NIEHS-M-001. Briefly, the mutagen assay involves exposing a culture of $TK^{+/-}$ L5178Y mouse lymphoma cells to the test compound, washing the cells, and allowing them to express any genetic lesions that might have been introduced. An identical

culture is sham treated as a negative control. Following expression time the cells are plated in soft agar cloning medium supplemented with -bromodeoxyuridine (BUdR); subsequent incubation kills off all but the $TK^{-/-}$ mutant cells which form visible colonies after 7-10 days. Viable counts, reconstruction using stock $TK^{-/-}$ mutants and positive (EMS or hycanthone) controls permit us to evaluate quantitatively the extent to which the given treatment has enhanced the mutation rate of these cells.

MAJOR FINDINGS: Classical mutagens have been used to correlate the response of our system with mutagenic hazard in whole animals. Thus 600 R of X-irradiation stimulates the $TK^{+/-} \rightarrow TK^{-/-}$ mutation rate 2500-fold over the spontaneous rate. The corresponding figures for the potent alkylation agent ethyl methanesulfonate (EMS, 10^{-2}M and 10^{-3}M) are 17,000-fold and 900-fold, respectively. Surprisingly, similar treatment with a populat antischistosomal drug, hycanthone methanesulfonate (10^{-4}M), resulted in a 450-fold stimulation of the mutation rate. This suggests that hycanthone methanesulfonate is about 5 times as mutagenic as the recognized classical mutagen D5.

Hycanthone, lucanthone and a number of hycanthone analogs (synthesized at Parke-Davis and at Sterling Winthrop) are currently under investigation for structure-activity relationships, as are a smaller group of alkylating agents. Mutagenicity data are evaluated at 2 or 3 different levels of sophistication: (1) As a function of external mutagen dose (concentration). This relationship ignores differences among drugs in their uptake, detoxification and interaction with DNA. (2) As a function of cell survival following treatment. Employing cell death as an index of how much mutagen interacts with the DNA falters on the assumption that a constant proportion of cell death is mediated by mutagen effects on the DNA over all compounds tested and for all concentrations. (3) For the alkylating agents, it is possible to quantitate the exact extent of chemical damage to the DNA (see Project No. NIEHS-M-___) because covalent reactions are involved; mutagenicity data can then be unequivocally expressed in terms of number of mutations induced per gene per alkylation. It is only this last evaluation which can accurately be considered mutagenic dosimetry.

While all compounds so far tested (hycanthone and 5 analogs; ethyl methanesulfonate, methyl methanesulfonate and ethylene dibromide) exert mutagenic effects in our system there have been quantitative differences in mutagenicity as a function of both mutagen concentration and mutagen toxicity. Thus we run the gamut from potent mutagens at low concentration and high toxicity, all the way through potent mutagens at high concentration and low toxicity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During the past century man has experienced the creation and dispersal of an unknown number of actual and potential mutagens, physical, chemical and biological. The genetic consequences of human exposure to such agents may range from severe first-generation effects to a gradual debilitation of the species over tens of generations. An alternative to such retrospective recognition of a mutagenic hazard is, of course, the early detection and control of environmental mutagens. Essential to such detection is a safety testing program utilizing a simple, sensitive mutational assay system, such as the one used here (see also Project Nos. NIEHS-M-004 and NIEHS-M-005). The demonstration of the mutagenicity of

hycanthone in mammalian cells has given additional impetus to safety testing at NIEHS. The studies on structure-mutagenic activity relationships are aimed at aiding in the development of a safe, efficacious anti-schistosomal drug, and in better understanding the molecular mechanism(s) by which these drugs exert their mutagenic effects. Both the alkylating agents and the hycanthone analogs are being studied to better evaluate the quantitative relationships existing between induced damage to the DNA and resultant mutant frequency (see Project No. NIEHS-M-___).

PROPOSED COURSE: Extension of the number and variety of hycanthone analogs and of alkylating agents used in this mutation assay will be augmented by similar studies employing mutants of varying genetic background (e.g., repair capabilities).

PUBLICATIONS

Clive, D., Flamm, W. G. and Machesko, M. R.: Mutagenicity of hycanthone in mammalian cells. Mutation Res. 14:262-264, 1972.

Clive, D., Flamm, W. G., Machesko, M. R., and Bernheim, N. J.: A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Res. 16:77-87, 1972.

Serial No.: NIEHS-MB-015
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Repair Replication of DNA in Opossum Lymphocytes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. Meneghini, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Repair replication of DNA is a cellular process which takes place when DNA is damaged by certain phy ical and chemical agents. Repair replication and normal semi-conservative replication are quite similar to each other as far as substrates and products of the reaction are concerned. Generally, repair replication accounts for only a small percentage of the total cellular DNA synthesis. This makes it very difficult to detect and measure. However, certain cell types (lymphocytes) which present a low level of semi-conservative DNA synthesis are very suitable for this purpose. It is our purpose to study repair synthesis in opossum lymphocytes. Basically, two major problems will be examined: (1) Many substances including carcinogens and mutagens can be tested in vitro for their capacity for inducing or inhibiting repair in lymphocytes; this may provide important data concerning the relationship between repair replication and mutagenesis or carcinogenesis; and (2) the in vitro and in vivo effect of these substances can be compared so that we can determine what role the whole animal plays in activating or inactivating some of these substances.

METHODS EMPLOYED: Lymphocytes in suspension are treated in vitro with the test agent and then incorporation of ^{3}H-thymidine is allowed for a certain period of time in the presence of hydroxyurea. This substance specifically inhibits the normal semi-conservative synthesis to an extent that permits the measuring of repair replication by simply determining the amound of thymidine incorporation into DNA. This incorporation can be ascribed to repair replication by two criteria; namely, autoradiography and CsCl gradient centrifugation of bromodeoxyuridine-labelled DNA. Autoradiography is useful because semi-conservative synthesis occurs in less than 1% of the lymphocytes while repair replication is

much more generalized. CsCl density centrifugation can be used because BUdR incorporation into DNA by repair replication process does not change the normal buoyant density of the DNA.

MAJOR FINDINGS: A cell suspension 60% enriched in lymphocytes can be prepared from opossum blood (the rest is constituted almost exclusively of red cells). In the presence of hydroxyurea the thymidine incorporation by these cells falls to very low levels so that small amounts of thymidine incorporation due to repair synthesis can be detected. A typical set of figures is: incorporation of ^{3}H-thymidine in the control: 262.6 cpm/µg; incorporation in the presence of 10 mM hydroxyurea: 9.9 cpm/µg; incorporation in the presence of 10 mM hydroxyurea after 1 hour incubation with 10^{-5} M proflavin: 33.3 cpm/µg. The reproducibility in duplicates is in general higher than 95%. Therefore, the assay is sensitive and accurate. Many drugs were tested at different concentrations to ascertain whether they induce repair replication in the lymphocytes. Positive results were found for two monofunctional alkylating agents (methyl methanesulfonate and ethyl methanesulfonate), two difunctional alkylating agents (Nitrogen mustard and ethylene dibromide) and a carcinogen (N-acetoxy-2-acetilaminofluorene). A group of substances known to bind to DNA through an intercalation mechanism were studied with special interest. These included actinomycin, proflavin, acridine orange, the antimalarial drug quinacrine, the antischistosome drug hycanthone and its N'-oxide derivative. Among these only the acridine, namely proflavin, quinacrine and acridine orange induced repair replication. It has not been proved whether repair replication is a direct consequence of intercalation or if it is due to the reaction of some metabolic product of the acridines to DNA. The former possibility is very interesting because it would mean that there is no necessity of covalent bond formation between the drug and DNA in order for repair replication to be induced. The possibility that these drugs act as inhibitors of repair replication induced by uv irradiation, with the exception of hycanthone N'-oxide. An inhibition of 50% was found in the range 10^{-6} to 10^{-5} M for quinacrine and in the range of 10^{-5} to 10^{-4} M for the others. However, none of them are specific inhibitors or repair replication because inhibition of semi-conservative replication was also observed in each case. The curves of drug concentration versus percentage of inhibition were essentially the same for repair and semi-conservative replications. The only exception was again hycanthone-N'-oxide which inhibits 50% of the semi-conservative replication in the range of 10^{-4} to 10^{-3} M, in spite of not inhibiting repair replication. Caffeine was also assayed in this system. Neither replication inhibition nor repair induction were observed in the concentrations as high as 10^{-3}M.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The assay here described provides the possibility of rapidly testing different drugs for stimulation and inhibition of repair replication. Both mechanisms could be implicated in mutagenicity and carcinogenicity. This assay can also be performed *in vivo*. In this case only the thymidine incorporation is carried out *in vitro*. This can be seen as a starting point for developing a similar study in man, with importnat clinical applications.

PROPOSED COURSE: Future efforts will focus on the survey of other drugs, most of them mutagens and carcinogens. There is special interest in obtaining a substance which acts specifically as an inhibitor of repair replication. This would be important both for the study of the relationship between mutagenicity and repair and as a powerful tool for repair study in cell-free systems. A comparison between the *in vivo* and *in vitro* results may bring valuable physiological information regarding the capacity of the organism to act on certain drugs.

Serial No.: NIEHS-MB-016
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of Normal Semiconservative Replication Patterns of DNA"

PREVIOUS SERIAL NUMBER: NIEHS-M-003

PRINCIPAL INVESTIGATOR: James B. Patterson, Ph.D.

OTHER INVESTIGATOR: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: A large number and variety of chemical compounds inhibit DNA synthesis and prevent the attainment of an appropriate G2 or 4n amount of DNA. If we examine the actions of antibiotic drugs alone on this cell function, we can construct an exhaustive list. These inhibitory actions may be divided into four basic types: (a) inhibition of de novo nucleotide synthesis; (b) inhibition of nucleotide interconversions; (c) inhibition of nucleotide utilization; and (d) nucleotide analog incorporation into polynucleotides. Undoubtedly some compounds affect the replication process in a subtle fashion that can lead to mutagenic events. Efforts to more fully characterize normal replication of the mammalian genome are based on the belief that such studies will aid in understanding the possible mutagenic effects of environmental agents such as certain antibiotic and alkylating agents.

METHODS EMPLOYED: Mouse lymphoma cells (L5178Y) can be blocked in metaphase with colcemid and held at the end of G1 with fresh medium containing 5-fluoro-deoxyuridine. Following reversal and the beginning of S-phase, DNA replication is assessed by incubating the cultures in medium containing 5-bromodeoxy-uridine. DNA is extracted and fractionated by isopycnic centrifugation in CsCl density gradients. Ribosomal RNA (rRNA) can be made highly radioactive and can be isolated essentially free of other RNA classes. Purification is achieved by first isolating cleas ribosomes, isolating RNA from them, then separating the RNS's on sucrose density gradients. With these fractions of DNA and r RNA, one can perform molecular hybridization experiments to determine

the point in time in S-phase at which replication of the ribosomal genes occurs. We are now implementing techniques (Pierre Chambon, personal communication) for the isolation of extremely high molecular weight DNA (400 x 10^6 daltons) for use in this work. Combining specific fractions of long-strand DNA with nucleic acid hybridization and electron microscope techniques which are now available allows one to determine the distribution of the genes coding for 18S and 28S rRNA in a mammalian genome.

MAJOR FINDINGS: The mouse genome replicates in such a fashion that the nucleotide sequences (genes) undergoing synthesis in early S-phase are of a different chemical composition from those genes or nucleotide sequences which replicate in middle of late S-phase. At the beginning of S-phase, only those nucleotide sequences which have a high guanine plus cytosine content undergo replication and, as replication proceeds, the guanine plus cytosine content of replicating sequences gradually decreases. From the beginning to the end of S-phase a change of 10% in guanine plus cytosine content is observed. It now appears that this is a general phenomenon applicable to, perhaps, all mammalian cells (including those of human origin); and while the underlying mechanism is not understood, these observations show that specific nucleotide sequences replicate at fixed times within S-phase. By introduction of BUdR-density label into newly replicated DNA, we can identify DNA which has been replicated at any chosen time in S-phase. We have isolated DNA from synchronized cultures in the appropriate time periods (early, mid, and late S-phase) and have separated newly replicated from non-replicated DNA. One class of genes which produce large amounts of an easily identifiable and isolatable product are the ribosomal RNA (rRNA) genes. We have succeeded in isolating ^{32}P-labelled 18S and 28S rRNA. In order to answer the questions we have posed, we now need to perform hybridization experiments. We have performed preliminary hybridization experiments and see no potential problems.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The above study has provided the basis for testing and evaluating the potential of environmental agents in altering normal patterns of DNA replication and may help explain the spectrum of biological and genetic effects produced by these agents.

PROPOSED COURSE: The above study indicates that specific genes are replicated at fixed times within S-phase which should enable us to isolate preparations of DNA which are greatly enriched in certain genes for transformation studies. We are presently attempting to learn when ribosomal and transfer RNA genes are replicated within S-phase.

Serial No.: NIEHS-MB-017
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Repair Replication of DNA in Mammalian Cells"

PREVIOUS SERIAL NUMBER: NIEHS-M-001

PRINCIPAL INVESTIGATOR: James B. Patterson, Ph.D.

OTHER INVESTIGATOR: Donald Clive, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.8
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The process that mediates gene repair must necessarily involve a set sequence of metabolic events. The first step involves the introduction of single-stranded breaks in DNA which facilitates the removal of the genetic lesion, such as the removal of an alkylated guanine. Secondly, the excised nucleotides are replaced by a new set of bases presumably in proper Watson-Crick register, through the function of a special repair enzyme or by the normal DNA polymerase. The last step in the process included a resealing (esterification) of 5' and 3' ends. It is our purpose to quantitate each of the above steps so that they may be related to data on damage to genetic material and induced mutation rates. We want to know how many single-stranded breaks are produced as each lesion is removed and the number of new nucleotides inserted in response to the event. We also want to assess the fidelity of repair and to learn whether this process influences mutation rates.

METHODS EMPLOYED: The process of repair replication in mammalian cells is difficult to measure by conventional techniques (i.e., incorporation of radioactive DNA precursors into DNA). This difficulty is due to the overwhelming masking effect caused by incorporation of nucleotide bases into DNA during normal semi-conservative gene replication. This problem can be attacked in practical terms by resolving the DNA molecules under going semi-conservative replication from those engated in repair synthesis by means of CsCl density gradients. There exist methods (e.g., treatment of cells with hydroxyurea or BUdR) by which normal semi-conservative synthesis can be selectively

reduced or stopped while repair synthesis proceeds in the presence of a radioactive precursor analog, BUdR. For practical purposes we use a combination of BUdR and ^{3}H-labeled thymidine.

MAJOR FINDINGS: Our main efforts are focused on delineating repair replication events in mouse lymphoma cells for which a mutational assay system is available. A previous study utilizing HeLa cells helped to provide direction and perspective toward the realization of this goal. Thus far, we have shown that monofunctional alkylating agents are capable of inducing demonstrable levels of repair replication in mouse lymphoma cells at concentrations known to mutate the TK locus. On the other hand, sulfur mustard, a bifunctional alkylating agent, does not induce a repair response. We have determined that in L5178Y mouse lymphoma cells 8×10^{-5} mutations are induced per locus per alkylation with ethyl methanesulfonate (EMS). This relationship between the numbers of alkylations and mutations remains constant over a five-fold range of EMS concentration. We are presently working to establish the quantitative relationship between the repair of these alkylations and genetic effects. The hydroxyurea system for reducing semi-conservative replication (NIEHS-M-001, 1972) has been extended to L5178Y cells. The HeLa cells previously employed display a much greater increase in repair synthesis in the presence of a given agent than do the mouse lymphoma cells (i.e., a 700% increase versus a 70% increase). Hydroxyurea is itself highly mutagenic, and the hydroxyurea system also allows the generation of false repair positive data by N-hydroxy compounds. For these reasons the simplified assay for quantitating repair replication is of limited utility for the current scope of our program. The laborious CsCl density gradient technique is more quantitatively precise and unambiguous in identifying repair-replicated DNA since it allows a physical separation of the two DNA populations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Repair of genetic damage before the onset of irreparable genetic alteration appears to constitute the primary means by which genes are properly maintained and protected from harmful environmental influences. Employment of the above system and others in which each step of repair is individually assessed and related to the overall process should reveal which chemical and physical agents interrupt and uncouple the sequence of repair events. Such interruptions could conceivably cause more damage to the cell's genetic material than the initial lesion. For instance, repair enzymes might produce multiple breaks in DNA and yet be prevented from resealing them by exogenous, environmental influences.

PROPOSED COURSE: Future efforts will focus on the relationship between genetic damage, repair replication, and the mutagenicity of the specific chemical agents employed in the L5178Y system. Correlations will be made utilizing a series of chemicals which should provide definite information as regards the underlying molecular mechanism responsible for mutation in mammalian somatic tissue. We further hope to be able to examine and characterize lines of cells which are repair-incompetent or repair-deficient.

Serial No.: NIEHS-MB-018
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Dosimetry of Chemical Mutagens"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: J. B. Patterson, Ph.D., D. Clive, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Before we can deal fruitfully with the important question of how mutations are mediated in mammalian cells by chemical mutagens, we must first attempt to answer another question. That is -- what is the quantitative relationship between the number of lesions introduced into genetic material and the number of mutations which result from exposure to that agent? We are attempting to answer this two-part question in two distinct operations. We first have to obtain data on a variety of known chemical mutagens in order to compare the relative mutagenic efficiencies (RME) of the compounds. We also must determine to what extent these compounds have an affinity for and a tendency to react with DNA. We wish to ultimately determine, for example, why only one EMS-induced alkylation out of about 10,000 results in an observable mutation in L5178Y mouse lymphoma cells.

METHODS EMPLOYED: Our operations are divided into two specific areas, (1) mutagen testing in mammalian cells and (2) measurement of the association of mutagens with DNA on a molecule per nucleotide basis. The mutagen testing methods are described in detail in NIEHS-M-004. The extend of DNA-mutagen interaction is measured by the following methods: (1) directly by the exposure of cells to radioactive alkylating agents, (2) by spectral perturbation during the interaction of DNA with various planar, heterocyclic compounds (e.g., hycanthone, and its analogs), and (3) the kinetics of depurination of DNA following interaction as described in NIEHS-M-002.

MAJOR FINDINGS: We have determined the dosimetric relationships for the potent alkylating agent, ethyl methanesulfonate (EMS) in L5178Y mouse lymphoma cells at the thymidine kinase (TK) locus. These are: (1) the number of alkylations per gene equals 6450 x $(EMS)^{1.27}$, where (EMS) denotes the molar concentration of EMS; (2) the induced mutation rate per gene per generation equals $(EMS)^{1.37}$; and (3) from equations (1) and (2) can be derived the dosimetric relationship where the mutation rate equals 7.8×10^{-5} x (the number of alkylations per gene)$^{1.08}$. This relationship holds over the 5-fold range of (EMS) that we have tested. Within the limitations of the techniques, the induced mutation rate in these cells and the number of alkylations per gene are proportional with a RME of 7.8×10^{-5} mutations per generation per alkylation.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In assessing the potential health hazard a given mutagen might present to man, a number of extrapolations must be made from the test systems. For our test system, these include: somatic vs. germinal cells; single cells vs. whole animals, and mouse cells vs. human cells. All of these factors represent potentials for ameliorating or enhancing the mutagenic effects of a given agent, especially by altering the extent of interaction between mutagen and DNA. By quantitating this interaction for various test systems, the confidence with which mutagenic data can be meaningfully extrapolated to man is greatly improved.

PROPOSED COURSE: We plan to extend these techniques to examine other alkylating agents (e.g., methyl methanesulfonate, ethylene dibromide). The MMS experiments are already in progress. If techniques can be perfected, we plan to examine other classes of chemical mutagens as well (e.g., hycanthone and its analogs).

Serial No.: NIEHS-MB-019
1. Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Internaciton of Environmental Agents with DNA"

PREVIOUS SERIAL NUMBER: NIEHS-M-002

PRINCIPAL INVESTIGATOR: J. B. Patterson, Ph.D.

OTHER INVESTIGATOR: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Certain classes of environmental agents interact either physically or chemically with DNA. Such classes include alkylating agents, certain N-hydroxy derivatives, nitrous acid and nitrites, bisulfites, certain analogs of purines and pyrimidines, a variety of antibiotics and mycotoxins, heavy metal ions and various polycyclic aromatic compounds containing either oxygen, nitrogen or sulfur in the ring system. In addition, other compounds such as polycyclic hydrocarbons (dimethylnitrosamine), heterocyclics (mitomycin C), and nitrosamines (dimethylnitrosamine) are metabolically converted to substances which attack nucleotide bases in DNA. It has been our purpose to devise a series of procedures by which the interaction of various environmental agents with DNA can be detected and the nature of the reaction products characterized.

METHODS EMPLOYED: Most mutagens that have been studied tend to react with purines to a much greater extent than they do with pyrimidines. Since the reacted purine is unstable, depurination occurs at that site, rendering the attendand phosphodiester bond alkaline labile. The rate to which bond lability occurs can be accurately assessed by sedimenting single-stranded DNA (alkaline) under strictly defined conditions of salt, pH and temperature. We have also employed a nitrocellulose filter technique whereby a denatured DNA sample is filtered allowing the released purine residue with its covalently-bonded radioactive alkyl group to pass through. Comparisons are made between counts remaining on the filter and counts in the filtrate. The two methods discussed here yield grossly comparable results, however, the sedimentation method is infinitely more sensitive to very low levels of depurination.

MAJOR FINDINGS: We have determined, both *in vivo* and *in vitro*, the rate and extent to which ethyl methanesulfonate reacts with DNA as well as the rate at which it depurinates. We have also shown unequivocally that depurination provides alkaline-labile sites which can be measured with exquisite sensitivity by band sedimentation analysis in the analytical centrifuge. This method is applicable to the study of a broad class of alkylating agents which have specific affinity for the N7 atom of guanine. We are currently extending our studies to methyl methanesulfonate. These data will be compared with mutagenicity data to obtain dosimetric measurements.

An alkylating agent which does not seem to have the same mode of action is 1,2-ethylene dibromide (EDB). EDB is a broad-spectrum pesticide used to fumigate tropical fruit. Traces of the chemical are found in marketed fruit. EDB has found such extensive use in the gasoline industry for the mobilization of tetraethyl lead that Dow Chemical Company developed a process for the extraction of bromine from sea water to meet increased demands. It is highly mutagenic in Neurospora (de Serres and Malling) and causes chromosome deletions. We have found that EDB is a potent alkylator but does not appear to lead to DNA breakage as does EMS. Preliminary experiments indicate that EDB is also a potent mutagen in L5178Y mouse lymphoma cells (D. Clive).

Only one compound examined so far has been found to be a stable alkylator of DNA. Lieberman and Dipple used bromo-methyl-a-anthracene, a potent carcinogen in non-dividing human lymphocytes, and found it to alkylate DNA at the 2-NH_2 site of G and the 6-NH_2 site of A. Both of these sites are involved in H-bonding and are not generally considered available for alkylation. If EDB alkylates at these sites, it might be expected to be both mutagenic and stable. We should consider that 6-NH_2-A and 2-NH_2-G alkylations are going to distort the steric relationship of the moieties of double-stranded DNA and will certainly affect any efforts at replication. Excision efforts by the cell's repair machinery could result in chromosome breakage. A stable alkylation product would be of great potential use in repair systems because elimination of the alkyl group is dependent fully upon enzyme systems because chemical breakage does not occur. If EDB does alkylate DNA in this manner, it may well be carcinogenic and might be an extremely interesting compound to examine in depth.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUE: In mammalian systems, all known mutagens appear to function by interacting with DNA, in many instances new covalent bonds are formed. By examining whether certain untested, environmental compounds and their metabolites interact with DNA and the conditions under which the reaction takes place, we derive quantitative information regarding their relative potential mutagenicity.

PROPOSED COURSE: Attempts will be made to utilize this highly sensitive procedure for determining whether compounds of great environmental importance have the potential for producing genetic damage. High on our list of compounds requiring study are fumigants and antischistosomal drugs.

Serial No.: NIEHS-MB-020
1. Mutagenesis Branch
2. Mammalian Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Comparative Sensitivities of Different Mouse Strains to Mutagenic Agents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: William Sheridan, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Earlier studies, itilizing irradiation as the mutagenic source, have shown that the genome of the test organism is a major factor in the determination of its susceptibility to genetic damage. It is our purpose to investigate to what extent such genetically caused differences in sensitivity pertain even to other mutagenic agents. We also want to know if the relative level of sensitivity of a defined genome to induction of mutations is constant for all agents or variable depending on the mutagen used.

METHODS EMPLOYED: We want to accrue a battery of test strains, each showing a distinct level of sensitivity to mutation induction, covering a range from highly sensitive to highly resistant. These strains will be selected on the basis of induction of dominant lethal mutations by a known potent mutagen (EMS). They will then be challenged by a successive array of ther known or suspected mutagenic agents to discover any changes in their relative levels of sensitivity or indications that suggest compounds may be mutagenic.

MAJOR FINDINGS: The project was initiated during March 1973.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because the gene pools of human populations by and large are extremely heterogeneous, one would expect a broad spectrum of reaction to a mutagenic agent in the environment. Restriction of testing to single, often inbred, strains of another mammal such as the mouse will not give indications on a wide scale of

what might be the effects of such an agent on the human population. By determining the effects of a compound on a battery of strains presenting a variety of genotypes and differing sensitivities we will better be able to approximate what might be expected in a heterogeneous mammalian population.

PROPOSED COURSE: Future efforts will be devoted to understanding the underlying genetic causes of differences in sensitivity to mutation induction, and the consequences of combining the genetic materials of strains with divergent sensitivities.

Serial No.: NIEHS-MB-021
1. Mutagenesis Branch
2. Mammalian Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Synergistic Effects of Radiation and Chemical Mutagens on Spermatogonia in Mice"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: William Sheridan, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Several investigations using known chemical mutagens have failed to show any indication of induction of dominant lethal mutations in the spermatogonial stem cells of mice. Studies with irradiation have shown that delivering a dose in two fractions increased the mutation rate, thus implying that the first fraction caused the stem cells to enter a more sensitive state by the time of the second fraction. It is our purpose to investigate whether pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemical treatments following the irradiation.

METHODS EMPLOYED: Adult male mice will be irradiated with 300 R of gamma rays. Twenty-four hours after irradiation they will be given a dose of a known potent chemical mutagen (EMS) and thereafter mated in dominant lethal dissection studies. The animals will go through a sterile period caused by the irradiation after which the germ cells tested will correspond to cells in the spermatogonial stem cell stage at the time of treatment. Results will be compared with those of animals receiving only irradiation or only chemical treatment as well as with those of a control group in order to determine any increase in the rate of dominant lethal mutations. Samples of males from all groups will be examined cytologically to determine the rate of induced chromosome aberrations.

MAJOR FINDINGS: The project was initiated during March 1973.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mutations occurring in spermatogonial stem cells are of major concern since they not only will deliver large numbers of germ cells carrying identical mutations but will do so for the remainder of the individuals fertile life. Knowledge of the degree of sensitivity of stem cells to mutation induction is pre-requisite to studies intending to estimate the risks from mutagenic agents.

PROPOSED COURSE: Future studies will investigate the effects of presenting chemical mutagens in fraction doses to determine whether there will be a sensitizing effect comparable to pre-treatment with irradiation. A cooperative program with biochemists will be entered upon to determine the underlying mechanisms of variation in sensitivity in stem cells.

Serial No.: NIEHS-MB-022
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Toxicity, Purification and Identification of Toxins Produced by Toxigenic Fungi"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: R. W. Pero, Ph.D. and Marion Blois

OTHER INVESTIGATORS: Charles Hodges, Ph.D., U.S. Forest Service

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	0.2
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the relative amounts of toxigenic fungi associated with health food and commercial food sources. These samples included wheat products, rice, corn meal, and flour.

METHODS EMPLOYED: A system of agar dilution plating methods was employed with a set amount of each sample. The resulting agar plates were incubated at 26°C and checked periodically for bacterial and fungal colony counts. The different species of fungi were then isolated. Each isolate was grown up on appropriate media, i.e., macaroni for wheat product isolates, rice for rice isolates, flour for flour isolates, and corn meal for corn meal isolates. After 14 days of incubation at 26°C, the cultures were extracted with 70% acetone. The crude extracts were screened for anti-bacterial activity (B. mycoides, S. aureus, and Sarcina lutea) and for anti-fungal activity (Helminthosporium satvium) measuring anti-germination, anti-mycelial mat formation, and anti-sporulation activity. The isolates showing either or both anti-bacterial or anti-fungal activity were further tested for tissue culture toxicity (Hela cells). The isolates found toxic to tissue culture were tested for mammalian toxicity in CD-1 mice at 200 mg/kg and 400 mg/kg.

MAJOR FINDINGS: In the rice, flour, and corn meal samples the colonies were either mainly bacterial (rice), high colony counts of the same fungal species (Fusarium moniliforme in corn meal), or of little or no toxicological interest (flour, corn meal, or rice). None of the health foods tested in these groups

showed significantly different numbers of bacterial or fungal colony counts or toxicological activity (bacterial, fungal, tissue culture, or mammalian toxicity).

The wheat product isolates showed a greater variety of isolate species and toxicological results. A total of 9 health food samples and 10 commercial samples were tested. The average bacterial colony counts (health foods 17, commercial 19) and average fungal colony counts (health food 2, commercial 5.5) at a 1:50 dilution were similar in both groups of samples. The major genera of fungi were of the most part similar to the two groups: Aspergilli, Penicillia, Mucors, and Rhizopus. Cladiosporium, Adsidi, Paecilomyces, Candida, and Streptomyces were also found but accounted for only 10% of the total number of isolates taken.

Although the anti-fungal and/or anti-bacterial activity of both groups was similar (72% in health foods, 70% in commercial), there were differences in toxicity in tissue culture (health foods 15%, commercial 29%) and in mouse toxicity. Aspergillus candidus isolates from health foods showed less tissue culture toxicity (9%) compared with commercial isolates (46%). In the A. flavus and A. flavus var columnaris isolates, there was one A. flavus in which TLC work indicated aflatoxin production. One A. flavus var columnaris isolate from commercial sources did exhibit anti-bacterial, anti-fungal, and tissue culture toxicity but in health foods this strain showed no toxicity. The Penicillia were more similar in tissue culture activity having only one species in each group exhibiting toxicity. In the A. glaucus group of isolates all types of toxicity were similar for the two groups. However, the most dramatic example of species differences was observed in A. terreus isolates. The health food isolate exhibited anti-bacterial and anti-fungal activity but no tissue culture or mammalian toxicity. The two commercial isolates showed both tissue culture and mouse toxicity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: The sample sources do indicate that the fungal populations of health foods are no more, if indeed less, toxigenic than commercial products. The toxicity in the human tissue culture system and in mice seem to indicate that health foods contain less toxic fungi than do comparable commercial products.

If this phenomenon were still supported after larger sample sizes have been evaluated, then alteration of our wheat products (pesticides, herbicides, vitamin enrichments, food cosmetics, etc.) tend to cause the fungi to adapt toward toxin production.

PROPOSED COURSE: There will be a limited amount of tissue culture screening and any resulting toxic isolates will be tested in mice. All animals injected with extracts of the isolates that have not or do not exhibit acute toxicity will be examined for chronic effects after a period of 6-9 months after injection. A more detailed study of the A. terreus isolates is planned to determine the active toxins.

Serial No.: NIEHS-MB-023
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Toxicity, Purification and Identification of Toxins Produced by Toxigenic Fungi"

PREVIOUS SERIAL NUMBER: NIEHS-CB-034

PRINCIPAL INVESTIGATOR: Ronald W. Pero, Ph.D.

OTHER INVESTIGATORS: Judson W. Spalding, Ph.D., and Donald Harvan

COOPERATING UNITS: Analytical and Synthetic Chemistry Branch

MAN YEARS

Total:	1.3
Professional:	0.5
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: During the past decade it has become increasingly clear that many fungi associated with food crops and processed foods produce toxic compounds under conditions favorable for their growth. Some genera, such as Aspergillus, Penicillium and Fusarium, because of their ubiquity and the high frequency of toxigenicity among species and strains, have been studied extensively. Others have received relatively little attention although evidence has been accumulated over several years that some of the lesser-known genera may also produce dangerous compounds. The objective of this study is to assess the potential hazard of some of these lesser-known genera and to identify the toxins they produce. Particular attention has been given to the genus Alternaria.

METHODS EMPLOYED: Toxigenic cultures were grown in large quantities either on rice fortified with Czapek-Dox broth or in other suitable culture media by standard mycological culture techniques.

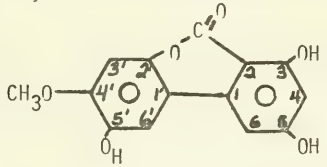
Extraction of toxins and other compounds from culture media and mycelia was done with a variety of solvents as deemed appropriate from our own experiences or those of other natural product chemists. One of the more useful solvents for initial extraction of the compounds reported here was 70% acetone in water. Generally, a secondary solvent fractionation was made with tetrahdyrofuran (THF) to obtain subfractionations that facilitated purification.

Separation of compounds and subsequent purification of the toxins were accomplished by column and thin layer chromatography on silica gel using various mixtures of benzene and THF. Toxicity of fractions was monitored by antibacterial assay (disc method) with Bacillus mycoides and antifungal assay with Helminthosporium sativum as indicator organisms.

Idnetification of purified compounds involved use of IR, NMR, MS and UV spectrometry and spectrophotometry and derivatization as necessary, and will, in some cases in the future, require x-ray analysis as point out below.

Mammalian toxicity of crude extracts and purified toxins was determined in random bred CD-mice, hybrid DBA mice and in cultured Hela cells.

MAJOR FINDINGS: A new compound, altenuene, was identified two years ago as a toxin from a culture of Alternaria tenuis. This year a second new compound was isolated. The compound has slower mobility on silica gel columns and plates than altenuene in the solvent systems employed. It was crystallized from hot acetic acid as buff-colored needles melting at 277-282°C. The mass spectrum indicated the parent molecular ion of 274, consistent with the empirical formula $C_{14}H_{10}O_6$. The UV spectrum and blue fluorescent emission under UV radiation were very similar to those of Alternariol (AOH) and its monomethyl ether. However, it differs from both in that it lacks the methyl group. The IR spectrum indicated the presence of all other functional groups that were present in AOH. The NMR spectrum in tetrahydrofuran-d indicated the presence of four aromatic protons (singlet 7.38 , doublet 6.92 , singlet 6.70 , doublet 6.47) and three methoxyl protons at 3.89 . A peak at 11.62 suggested a proton from a hydroxyl group hydrogen bonded to a carbonyl. The aromatic doublet protons were assigned to the unprimed ring as meta-coupled (structure I) and the singlets were located on the primed ring as uncoupled para-protons. The methoxyl group was placed at C-4' by comparison of I and its triacetate derivative with scopoletin (model system) and its acetate. Because the greatest dowfield shift was observed in the proton at C-6', then acetylation must have taken place at C-5'. Thus, the structure for I (3,5,5'-trihydroxy-4'-methoxy-dibenzo- -pyrone), trivally named Altenuisol, was considered correct as assigned.



I = Altenuisol

The ID_{50} dose of Altenuisol with Hela cells was 8 g/ml and zones of inhibition with Bacillus mycoides were noted from 200-5 g/assay disc.

Two new compounds designated Altertoxin I and Altertoxin II were isolated from Alternaria mali, originally obtained from apple and shown to be toxic to mice. The compounds were obtained in mixture by column chromatography on silica gel G, using THF-benzene mixtures with increasing proportions of THF. The mixture of subsequently resolved into two yellow compounds by re-chroma-

tography on silica gel G, using 30% ethyl acetate in benzene. Altertoxin II was crystallized as slender needles from DMSO-water and it fluoresced black under UV light. Altertoxin I has not been crystallized and it fluoresced bright yellow under UV light.

Mass spectrometry indicated molecular weights of 352 and 350 for Altertoxins I and II respectively, consistent with the empirical formulae $C_{20}H_{16}O_6$ and $C_{20}H_{14}O_6$. Apparently, Altertoxin II is the dehydro form of Altertoxin I. IR and NMR spectra were not sufficient for structural elucidation. An Altertoxin II crystal was grown large enough for collection of x-ray data. The data collected is sufficient for structural determination and is now being analyzed by various computer program systems.

Altertoxin I had an ID_{50} of 20 g/ml against Hela cells in culture and was lethal to 8 of 8 female mice at 200 mg/kg administered ip. Altertoxin II was highly active against Hela cells (ID_{50} = 0.5 g/ml) and killed 2 of 2 female mice at 200 mg/kg. More detailed toxicological data is being collected on the Altertoxins.

Some ten toxic isolates of Alternaria associated with grains were obtained from Christensen and his colleagues at the University of Minnesota. Crude extracts from these isolates were all analyzed for the presence of known Alternaria metabolites. They all contained Alternariol and its monomethyl ether, Altertoxin I, and tenuazonic acid. In no cases, could the animal toxicity of the biologically active purified metabolites explain the toxicity observed with crude extract preparations. This suggested a synergism between the biologically active components.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Work over the past decade has clearly demonstrated the potential hazard of certain molds associated with agricultural commodities. The frequency of reports of new toxins from many genera of fungi is indicative of the paucity of information about fungi that inhabit food products and the nature of the toxins they produce. It is essential, therefore, that efforts continue toward better assessment of the potential importance of fungal byproducts in foods and their long range effects, in combination with other chemical agents, on the health of man.

PROPOSED COURSE: Efforts to isolate, purify and identify toxins from significant toxigenic fungi on hand, from out screening program and from other sources will continue. In concern with this, efforts will continue to develop fuller understanding of the toxicological aspects of both known and new mycotoxins.

Serial No.: NIEHS-MB-024
1. Mutagenesis Branch
2. Microbial & Plant Genetics Sec.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Analytical Methodology for Mycotoxins"

PREVIOUS SERIAL NUMBER: NIEHS-CB-035

PRINCIPAL INVESTIGATOR: Ronald W. Pero, Ph.D.

OTHER INVESTIGATORS: Donald Harvan

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To develop reliable qualitative and quantitative methods for detection and measurement of mycotoxins in naturally moldy agricultural commodities, foods and mammalian fluids and tissues.

METHODS EMPLOYED: Development and refinement of analytical methodology for a number of mycotoxins was continued. Basically, the procedures were centered around partition between solvents, thin layer chromatography and gas-liquid chromatography.

MAJOR FINDINGS: An analytical method for Altertoxin I was established using a fluorodesitometric assay. A Zeiss thin layer chromatogram spectrophotometer with a Hg-line excitation lamp was used. The Altertoxin I spot was irradiated with monochromatic light using an M-365 filter. The emission was measured at 534 mμ at a slit setting of 0.60. Concentration of Altertoxin I was linear to the percent transmission between 0.1 - 5.0 μg. Crude extracts containing Altertoxin I were chromatographed on silica gel G (250μ) plates using 30% ethyl acetate in benzene. The area of the chromatogram corresponding to Altertoxin I standard was removed, eluted with tetrahydrofuran and rechromatographed in the same solvent system. Recovery from thin layer plates was determined to be 70%.

A gas chromatographic method for the simultaneous detection of twelve fungal metabolites has been developed. The metabolites, listed in order of increasing retention time are: succinic acid, erythritol, penicillic acid, terrein,

patulin, kojic acid, mannitol, palmitic acid, stearic acid, altenuene, alternariol and alternariol monomethyl ether. The method involves silylation N,O-bistrimethylsilyl acetamide (BSA):trimethyl chlorosilane:pyridine,6:2:9% and chromatography on 3% OV-17 with a temperature program of 100-250° at 8°/min. A method for tenuazonic acid has been worked out using the same column packing and temperature program, but its retention time is similar to several of the aforementioned metabolites and so cannot be co-chromatographed. The BSA silyl reagent is critical for complete silylation of tenuazonic acid and its salts.

The above methods were applied to extracts of naturally moldy commercial tobacco samples obtained from N.C. State University. AOH, AME, altenuene or patulin were not detected in samples previously analyzed. However, penicillic acid (PA) was detected in tobacco naturally infested or artificially inoculated with Aspergillus ochraceus and Penicillium cyclopium. Naturally moldy tobacco contained 10 to 20 µg PA/100g of tobacco. Levels in artificially inoculated tobacco reached 320 to 1800 µg/100g. It was shown also that as much as 3% of PA in tobacco made into cigarettes and smoked could be recovered in the smoke condensate. This finding prompted contract support of further research at N.C. State University on the occurrence of PA in tobacco and in cigarette smoke. The contract was initiated January 1, 1972, and will continue through August. Determination of tenuazonic acid in tobacco is now underway.

PROPOSED COURSE: Efforts will continue toward development and refinement of sensitive quantitative analytical procedures for new toxins as they are found and for known toxins where existing procedures are inadequate.

PUBLICATIONS

Snow, J.P., Lucas, G.B., Harvan, D., Pero, R.W. and Owens, R.G.: Analysis of tobacco and smoke condensate for penicillic acid. Appl. Microb. 24(1): 34-36, 1972.

PATHOLOGIC-PHYSIOLOGY BRANCH

PATHOLOGIC PHYSIOLOGY BRANCH
Summary Statement

The scientific efforts of this Branch are generally directed toward determining the mechanisms by which environmental agents alter gametogenesis, reproduction, ontogenesis and development. These basic studies will hopefully lead to the development of test methods to predict reproductive toxicity that will be more rapid, reliable and inexpensive than those currently available. Test systems are diverse and range from subcellular biochemistry and electron microscopy, through in vitro cell cultures to whole animals. Attempts are made to support strong independent basic research programs in interrelated areas which have a direct application to current toxicologic problems in environmental health.

The Pathologic Physiology Branch conducts research in three major sections: (1) Gametogenesis and Fertility, (2) Reproduction and Ontogenesis and (3) Biochemical Toxicology. Within this organizational strauctured eight "workgroups" are defined and represent, in some cases, projects which are being de-emphasized in the future to provide resources for research efforts which have a greater priority and relevance to this Institute. The "workgroups" are (1) Gametogenesis and Fertility, (2) Reproduction and Ontogenesis, (3) Biochemical Toxicology, (4) Mammalian Tissue Culture, (5) Heavy Metal Toxicology, (6) Maternal-Fetal Distribution, (7) Marsupial Program and (8) Membrane APTase, and each will be described individually.

Gametogenesis and Fertility. Doctors Lee, McLachlan and Dixon have extended their studies of the effects of anticancer agents (initiated at the NCI) on spermatogenesis and oogenesis to include the study of environmental chemicals.

The velocity sedimentation cell separation technique is being utilized to separate spermatogenic cells of the seminiferous tubules. The effects of in vivo treatment or in vitro exposure with environmental chemicals on biochemical parameters of individual cell types are then studied. Attempts are made to correlate biochemical alterations induced by chemicals to in vivo changes in male fertility as determined by serial mating. Studies are also in progress to define the development of selected enzymes during spermatogenesis in order to develop useful "marker" enzymes which can indicate specific cell types, cellular differentiation and/or toxic effects. More recently, studies have been initiated to investigate the nature of the "blood-testicular barrier" and to elucidate the importance of this "barrier" in the penetration of chemicals to male spermatogenic cells.

Other studies are attempting to evaluate the effects of prenatal exposure to foreign chemicals on the subsequent reproductive capacity of the offspring. Very low single doses of cyclophosphamide or procarbazine on day 10 or 12 of gestation result in a significantly decreased reproductive capacity of female offspring. The mechanisms involved in the production of subfertility as a

result of in utero exposure to foreign chemicals are being further investigated. The carcinogenic potential of these gestational treatments is also being studied. More recently, studies to define the distribution of compounds between blood plasma and adult or fetal ovarian compartments have been initiated, as well as attempts to define and compare the embryonic and/or postnatal period of DNA synthesis of germ cells for various experimental animal species.

An important new project in this general area is an attempt to grow embryonic germinal tissue and adult spermatogonia in in vitro culture. Doctor Spalding is playing an important role in this new area.

Reproduction and Ontogenesis. This effort is headed by Doctor Staples and involves research to develop methods for assessing the teratogenic potential of given environmental compounds that will have greater predictive value for man. Selected environmental components to which young women are exposed and which might contribute to reproductive or teratogenic risk through individual action or through chemical interaction are being studied.

The practice of administering test agents across species to an arbitrary mg/kg dose level, or to a given multiple of the proposed human dose is all too common. Hopefully, interspecies variability can be limited by selecting dose levels on the basis of a pharmacological response, thereby permitting subsequent interspecies comparison after equating susceptibility differences among species to the test agent. The doses required to produce a given endpoint after administration to the dam are also compared to those doses which are effective after intrauterine application of the test agent. These experiments should enhance the sensitivity of the tests, and the quality of the response might be made more uniform among species.

As an additional refinement for circumventing the maternal influence, the agent under test is applied directly to the embryo of each species by adding it to the gaseous or liquid environment of the embryo during culture, or by direct injection into the blastocyst through application of a technique previously developed by Doctor Laila A. Moustafa. Effects on subsequent development are assessed following transfer of the embryos to the uteri of foster dams. The results obtained in vitro and in vivo are compared with respect to relative susceptibility among species tested and degree of qualitative difference. These approaches are being evaluated by studying classical teratogens, e.g. thalidomide and DMSO.

Biochemical Toxicology. This section is studying a variety of enzymes which are important in regard to environmental toxicology. Hopefully, these basic studies will further the understanding of toxic mechanisms of action and allow for the development of more predictive test systems. This effort is headed by Doctor Voytek who is investigating whether thymidine kinase activity in various thymidine kinase mutant cells is caused by mutations in control or structural genes. The thymidine kinase locus in L5178Y mouse lymphoma cells grown in tissue culture is an important assay for mutagenic potential. Doctor Woods is attempting to elucidate the mechanisms governing the regulation of hepatic hematopoiesis during mammalian development through analysis of hepatic δ-aminolevulinic acid synthetase (ALAS) activity. ALAS is an important rate-limiting enzyme in prophyrin synthesis which is essential to a variety of heme

containing enzymes, e.g. cytochromes. Doctor Weissberg is attempting to characterize changes in red cell ALA dehydratase during development. ALA dehydratase is important in porphyrin and heme synthesis, and is affected by lead. Doctor Crapo is investigating the relationship between the level of superoxide dismutase (SOD) present in lung tissue and the ability of an animal to survive oxygen (free radical) toxicity. A large number of environmental toxicants including ozone, NO_2 and paraquat produce part of their toxic effect by way of the superoxide free radical, and the current studies with SOD and oxygen may have a broad application in this area.

Mammalian Tissue Culture. Doctor Spalding is in the process of completing his studies of the toxicity of pesticides and other xenobiotics to cells in culture and the study of mechanisms by which cultured cells become resistant to chemicals. The research efforts of this group are being directed toward the development of methods and techniques for establishing in vitro tissue or organ cultures of mammalian gonads. These in vitro cells will be used to investigate mechanisms by which chemicals affect oogenesis and spermatogenesis. Doctor Spalding is collaborating with Doctors Lee, McLachlan and Dixon with regard to the culturing of germinal tissues and/or cells.

Heavy Metal Toxicology. Doctors Fowler and Klein have continued a variety of studies of the toxic effects of heavy metals. These studies involve the clinical, physiologic, metabolic, biochemical and morphologic effects of heavy metals on experimental animals. Agents are administered singly or in combination with other environmental chemicals either acutely, subchronically or chronically. The in utero effects of heavy metal exposure have also been studied. Special emphasis is placed on methylmercury and mercury, cadmium and arsenic. This research group has been particularly productive.

Maternal-Fetal Distribution. Doctor Posner has been directing a research effort which is concerned with the passage of toxic environmental chemicals across the placenta and the comparative accumulation of the chemical in fetal and maternal tissues. The interaction of the test chemcial with other agents administered concomitantly is being investigated. An attempt has also been made to compare the events that occur in the fetal rat and/or mouse during gestation in association with cleft palate formation, and to test the role of water balanne (edema) in the production of this defect.

Marsupial Program. Doctor William Jurgelsky, with support from the Animal Science and Technology Branch, has contined to develop the opossum as a Marsupial model system which will be useful in the investigation of a large variety of physiologic, pharmacologic, pathologic and toxicologic mechanisms. The Marsupial is at the inframammalian position in the evolutionary scale, and is endowed with a number of unique developmental characteristics. Doctor Jurgelsky has continued a variety of investigations which include the potential uses of the Marsupial neonate as a model for the identification and evaluation of environmental toxicants and carcinogens; an evaluation of the toxicity of sex hormones to the opossum; chemical and morphologic studies of the role of lipid metabolism in CNS irritability and seizures; the effects of iodine and iodine deficiency on the endocrine system of the opossum; and the development of tumor models.

Membrane ATPase. Doctors Wilson and Sharp have continued their attempts to determine the mode of action of chlorinated hydrocarbons (DDT and PCB's) and of a variety of detergents and inhibitors of the ($Na^+ + K^+$) ATPase system from several tissue sources. An understanding of the mode of toxicity of the chlorinated hydrocarbons with regard to these enzyme systems would permit the development of a model to assess the toxicologic significance of enzyme alterations which result from exposure of a mammalian cell to these environmental chemicals. These studies would also provide a more comprehensive understanding of the phospholipid dependence of ($Na^+ + K^+$) ATPase systems in various mammalian plasma membranes.

The Pathologic Physiology Branch will continue during the coming year to support solid independent research programs and scientists capable of designing and conducting experiments which test relevant hypotheses and culminate in the publication of results in the scientific literature. A major problem in the past year has been the relative lack of productivity associated with well-supported and expensive research efforts. The coming year should document an improvement in these areas as a result of changes in Branch emphasis and support.

Serial No.: NIEHS-PP-002
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Effects of Excess Iodine and Iodine Deficiency on the Endocrine System of the Opossum (Didelphys marsupialis virginiana Kerr)"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Paul J. Davis, M.D., Baltimore City Hospitals, and Pearlie M. Hudson

COOPERATING UNIT: Endocrinology Division, Baltimore City Hospitals, Baltimore, Maryland

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Work performed elsewhere by members of this laboratory has revealed that the adult opossum develops a flagrant intrafollicular thyroiditis in response to the administration of potassium iodide at levels within the therapeutic range of humans. The implication from these studies, as well as from those of other workers who have demonstrated a low PBI in the opossum and a low thyroxine secretion rate, is that the marsupial has an iodine transport and storage mechanism that differs markedly from that of other mammals and is thus worthy of evaluation as a potential model for studies of the role of iodine in the endocrine system. The objective of these studies is to define the structural and chemical changes that take place within the hypothalamic-pituitary-thyroid axis of the opossum in response to iodine excess and iodine deficiency.

METHODS EMPLOYED: Through the use of column chromatography, thin-layer chromatography and scintillation spectroscopy, in vivo and in vitro radioiodine metabolism is being studied in adult opossums treated with potassium iodide. Histological changes are being documented by light and electron microscopy. Thyroid hormone binding is studied in a polyacrylamide electrophoresis system.

MAJOR FINDINGS: An initial study of the T_4-distribution in opossum serum (with Dr. P. J. Davis) has been completed. In a population of 177 animals surveyed with a polyacrylamide electrophoresis system, it was found that thyroid hormone binding in the opossum is limited to postalbumin and to serum albumin. The binding protein pattern in the opossum is in sharp contrast to the Australasian marsupial, the kangaroo, which (like man) shows prealbumin, albumin and postalbumin binding of T_4 and in which prealbumin is the major carrier. This work has also revealed a polymorphism of the postalbumin and of the haptoglobin factions. The close relationship between the appearance of uncommon postalbumin and haptoglobin phenotypes may represent genetic linkage or possible overlap of functions of hemoglobin and T_4-binding by a serum protein. Serum albumin also appears polymorphic in the opossum.

These studies provide evidence for a second instance of T_4-binding protein polymorphism in the mammal (the initial report of this phenomenon was in the rhesus monkey) and, for the first time, reveal an interrelationship between protein binding of T_4 and haptoglobin. These findings are in press and data from the excess iodide study are in the process of summarization.

SIGNIGICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The intricacies of the role of iodine in the mammalian endocrine system are largely unknown. Such knowledge is essential to an understanding of many endocrinopathies, including thyroiditis, hyperthyroidism and hypothyroidism and for evaluation of the possible role of environmental contaminants in these pathologic processes. Study of iodine metabolism and thyroid function in an animal such as the opossum in which response to iodine appears anomalous could yield insight into mechanisms of iodination, iodine transport and iodine storage.

PROPOSED COURSE: These studies are terminated due to withdrawal of support.

PUBLICATIONS

Davis, P.J., and Jurgelski, W.: Thyroid hormone binding in opossum serum. Evidence for polymorphism and relationship to haptoglobin polymorphism. Endocrinology. 92:822-832, 1973.

Serial No.: NIEHS-PP-003
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Chemical and Morphologic Studies of the Role of Lipid Metabolism in CNS Irritability"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Pearlie M. Hudson; Clyde Boyd; Frederick Snyder, M.D., NIMH; and Michael O'Connor, NINDS

COOPERATING UNTIS: Section on Electroencephalography, NINDS
Laboratory of Psychobiology, NIMH

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Disorders of steroid metabolism have been implicated among the causes of epilepsy. Previous studies, performed elsewhere by members of this laboratory, have established that an androgen-related, cholesterol-biosynthesis inhibitor, when administered once weekly to young marsupials (Didelphys marsupialis virginiana Kerr) in doses well below the toxic levels, will induce a chronic major motor seizure state. Seizures may be induced at will by tactile stimulation. The opossum alone, among those standard laboratory species thus far tested, develops CNS hyperirritability in response to this drug. A number of organic phosphates and chlorinated hydrocarbon pesticides, which are known to influence steroid metabolism, also produce seizures at high levels of exposure. In the studies underway alterations in lipid metabolism produced by this drug and other agents, such as pesticides with seizure-producing potential, will be correlated with changes in CNS irritability.

METHODS EMPLOYED: Optical microscopy, electron microscopy, thin-layer chromatography, electroencephalography, ultra centrifugation, gas chromatography and scintillation spectroscopy are being used. Comparative studies are being performed in rats and opossums.

MAJOR FINDINGS: The available data are in the process of summarization. One manuscript has been submitted for publication and a second is in preparation.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The induction of seizures in the marsupial by administration of an inhibitor of cholesterol biosynthesis represents a previously-unreported easily reproducible model for experimental chronic epilepsy. The apparent direct implication of disordered steroid biosynthesis in the seizure state in these animals may offer a new window on neural irritability in general and on epilepsy in particular.

PROPOSED COURSE: Due to lack of indicated support, this study will be terminated when surviving animals die. Attempts to further characterize the malabsorption syndrome associated with triparanol administration described in Annual Report of July 1, 1971 to July 1, 1972 cannot be completed.

Serial No.: NIEHS-PP-004
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: M. E. Porter, P. M. Hudson, C. W. Boyd and G. L. Stranzinger, Ph.D.
J. Zinkl, Ph.D., and J. E. Thigpen, Ph.D., Animal Science and Technology Branch
H. B. Matthews, Ph.D., Pharmacology-Toxicology Branch
W. Wood, Ph.D., J. Massicot, C. Dawe, M.D., M. Chirigos, Ph.D., and J. Rice, Ph.D., NCI
A. Moore, P. Mortillaro, J. Romine and A. Godwin, MAPB
R. Martensen, Ph.D., NIMH
Alfred Hellman, Ph.D., NCI
G. Alderson, D.D.S., M.D., C. Boyers, D.D.S., L. Zimmerman, M.D., K. Ishak, M.D., F. Mostofi, M.D., L. Hockholder, M.D., and L. Johnson, M.D., AFIP
J. Sommers, M.D., I. F. Brody, M.D., Daryl Bigner, M.D., and J. Wagner, D.V.M., Duke University Medical Center
U. J. Lewis, Ph.D., Scripps Institute

COOPERATING UNITS: Animal Science and Technology Branch
Pharmacology-Toxicology Branch
Laboratory of Pathology, Viral Leukemia and Lymphoma Branch, Experimental Pathology Branch, and Viral Oncology Branch, NCI
Medical Arts and Photography Branch, NIH
Laboratory of Cerebral Metabolism, NIMH
Oral, Ophthalmologic, Orthopedic, Hepatic, Renal and Pulmonary Branches, AFIP
Animal Resources, Neurology and Pathology Departments, Duke University Medical Center
Scripps Institute, LaJolla, California

MAN YEARS

Total:	7.0
Professional:	3.0
Other:	4.0

OBJECTIVES: The opossum (Didelphys marsupialis virginiana Kerr) has a number of unique characteristics which make it of interest as a biomedical model. These include (1) absence of chorio-allantoic placenta; (2) gestation period of 12-3/4 days; (3) birth at a stage of development roughly equivalent to an eight-week-old human or an eleven-day-old rat *in utero* with completion of development in an extrauterine environment, the pouch, permitting experimental manipulation of developing embryonic and fetal tissue in the absence of placental and maternal metabolic interference; (4) markedly slow development of the nervous, respiratory and endocrine systems over a three-month postnatal period; (5) anomalous metabolic responses to several drugs and toxins (iodide, estrogen); (6) tolerance of developing young to high CO_2 levels and (7) rapid growth rate during the first year of life. The experimental possibilities which these attributes offer have not been exploited to any degree due to an inability to breed the opossum in captivity and to the lack of suitable methods for laboratory manipulation of the animal.

The objectives of this study are (1) to develop techniques for efficient breeding of the opossum under laboratory conditions, (2) to develop methods of care for the adult animal and techniques for experimental manipulation of the adult and neonatal opossum and (3) to characterize the neonate and developing opossum anatomically and physiologically and to determine their pathophysiologic responses to selected environmental toxins and carcinogens.

METHODS EMPLOYED: Special pharmacological techniques, light and electron microscopy and standard biochemical methods of ultracentrifugation, thin-layer chromatography and gas chromatography.

MAJOR FINDINGS:

(1) Opossum Breeding. The opossum breeding facility and the breeding techniques which we have developed have been described in two previous annual reports. Reproduction data derived from the use of these procedures during two successive breeding seasons (1970-1971 and 1971-1972) are summarized below. These data are based on a total population in each season of 130 females. (a) The total number of litters produced was 103 in 1970-1971 and 124 in 1971-1972. Fertility was 68 and 70 percent in respective years and total birth rate was 79 and 96 percent, respectively. Fertility in females captured prior to estrus was 30 to 40 percent higher than in females captured after estrus onset. Mean litter size was 8.3 and 7.9 in the two years. The fertility and litter size figures available compare favorably with those reported for wild populations implying that our system of maintenance and breeding does not, for the first time, adversely affect the reproductive performance of the opossum in captivity. In all opossum breeding colonies described during the past 80 years, fertility has been either erratic or greatly reduced and litter sizes have been smaller by comparison with the wild population. (b) The birth of multiple litters (up to four) in 26 out of 129 females in 1971-1972 implies that the maximum birth rate has not yet been attained under our conditions. Data obtained in the two successive seasons indicate that the reproductive life span of the female opossum is one year dictating the necessity of turning over the colony annually if an acceptable breeding efficiency is to be obtained. (c) Data summarized to date show that the mean length of the estrus cycle under our conditions was

25.1 days. (d) An incidental finding of interest was a high incidence of trichomonas infections in females in the breeding colony. This observation has led to a consideration of means of therapy to eliminate possible deliterious effects on reproduction and interpretation of vaginal cytology. (3) Descriptions of the breeding facility and methods for controlled breeding are in press. Two additional manuscripts on opossum reproductive biology and the opossum estrus cycle respectively are in preparation.

(2) Development of Laboratory Methods. A description of techniques developed for care and laboratory manipulation of the adult and neonatal opossum is in press. These techniques will also be illustrated in the film in preparation described below.

(3) Opossum Film. A total of 39,000 feet of film has been exposed in the production of a film entitled "The American Opossum as a Biomedical Model: I. The Neonatal and Developing Opossum, II. The Adult Opossum". This film will cover opossum biology and experimental uses and will depict in detail the several special experimental techniques which we have developed for the opossums. Several sequences remain to be filmed before editing can begin. However, the completion of the work (originally scheduled for Spring, 1973) has been delayed by refusal of the committee appointed to review opossum usage to make necessary animals available. The animals were originally requested in November, 1972. While this decision has recently been overruled by the Director, our ability to complete the requisite sequences will depend on availability of young in the waning portion of the breeding season. Further, our ability to properly edit the film and to complete it for release will depend upon continued support by NIEHS. If this is terminated, extra-NIEHS, and, if necessary, extra-NIH funds will be sought in order to finish the film.

(4) Biologic Characterization of the Opossum. The following collaborative studies were initiated in FY 1971 and 1972. (a) The isolation and characterization of growth hormone and prolatin from the opossum pituitary and the development of immunoassay for opossum growth hormone with Dr. Lewis, Scripps Institute, La Jolla, California. Pituitaries from 55 opossums have been provided for this work. (b) A study of the morphology and myotogenic potential of the skeletal muscle of the opossum with Dr. Brody, Department of Neurology, Duke University Medical Center. We have found that the opossum, unlike other mammals thus far tested, does not develop myotonia from parenteral administration of 2,4-dichlorophenoxy acetic acid (2,4D). In this response opossum skeletal muscle is like frog skeletal muscle or mammalian cardiac muscle. Further work has revealed that phosphatase levels in opossum skeletal muscle exposed to 2,4D do not differ from rat skeletal muscle showing myotonia. Further studies with other myotogenic agents and additional biochemical characterization of the normal and treated opossum skeletal muscle have been terminated by decision of the committee described above. (c) Light and electron microscopic studies of developing opossum skeletal muscle with Dr. J. Sommers, Department of Pathology, Duke University Medical Center. It may be of some significance in the anomalous response described in Item b above that the opossum skeletal muscle is morphologically like mammalian cardiac muscle for two weeks postpartum. Techniques of sample preparation have been developed but further efforts have been terminated prematurely by

decision of the committee described above. (d) Isolation and electrophoretic characterization of brain protein in the opossum as part of the phylogenetic comparison underway in the laboratory of Dr. R. E. Martensen, Laboratory of Cerebral Metabolism, NIMH. Brains from over 100 animals at various stages of development are currently being processed for this study. (e) A pilot study to determine levels of mixed function oxidases and UDP glucuronyl transferases in the livers of developing opossums with Dr. H. B. Matthews, Pharmacology-Toxicology Branch. Results in four animals were equivocal. This work has been terminated by decision of the committee and Institute reorganization. (f) Isolation and characterization of viruses from wild weeks of age (two tumor bearing animals are present in a single litter). These lesions represent the first intraoocular tumors ever induced in a laboratory animal by systemic exposure to a carcinogen and appear to offer much promise as a model for intraoocular carcinogenesis. (c) About 85 percent of autopsied animals treated at all ages from birth to 16 weeks have had nephroblastic tumors. These tumors contain a high proportion of tubular and glomerular components and thus are morphologically much more comparable to human nephroblastomas than those induced in other laboratory species to day. Again, this readily inducible tumor in the opossum promises to be of considerable value as a model for study of nephroblastomas--a tumor with the fourth highest incidence of all tumors of childhood. (d) Approximately 13 percent of all treated animals coming to autopsy have had tumors of the oral cavity. Tumors have been induced in animals exposed at all ages through 16 weeks. About 5 percent of the tumors are odontogenic (ameloblastic) tumors. The remaining animals with oral tumors have a variety of malignant tumors including tumors of the salivary glands, gums and hard palate. Oral tumors have arisen both in animals whose oral mucosa was directly exposed to the carcinogen and in those given the drug by direct intragastric administration. There is only one other report of the successful induction of odontogenic tumors in a laboratory animal, the hamster. However in that study odontogenic tumors were limited to incisors which are continuously growing teeth. (e) Approximately 50 percent of all autopsied animals treated at ages from birth to 16 weeks have had tumors of the liver. About 25 percent of these tumors are either fibrosarcomas or hemangiosarcomas; the remainder are hepatomas. Other sites of primary tumors have included bone thyroid, thymus, lung, pinna, scrotum, penis, ovary, skin, tongue, stomach, small intestine, large intestine and brain. The incidence of brain tumors (about 2-5 percent) is extremely low by comparison to that (over 90 percent) in the rat exposed to ENU in utero by intraperitoneal administration to the pregnant and captive opossums with Dr. Thigpen, Animal Science and Technology Branch. Brain and serum samples from 55 wild captured opossums representing a portion of the 1972 breeding colony are being made available to the collaborator. Selected tumor tissues from animals treated with ethyl nitrosourea is also being forwarded to Dr. Thigpen for possible virus isolation. In addition to the above collaborative studies, two reviews are in preparation on the biology and available experimental data of the adult and neonatal opossum. They will be submitted to appropriate journals and will also be included in the serial of chapters in preparation for the book Biology of the Marsupial to be published by Academic Press.

(5) Carcinogenesis Study. (a) An experiment to determine the response of the opossum at 10 stages of development (birth to 16 weeks) to the highest

tolerated (100 mg/kg) single oral dose of ethyl nitrosourea continues. Sacrificing of animals assigned to the first phase of this study will be complete in September 1973. Histologic preparation and interpretation will require an additional six months to one year. (b) Results to date indicate that the opossum exposed to ethyl nitrosourea as described above has a unique capacity to develop several types of tumors not previously induced in laboratory animals. For example, thus far, five malignant introccular tumors (teratoid medulloepitheliomas) arising from the ciliary body and retinal elements have been observed. The incidence of these tumors is about 20% in the population treated either at birth, one week, two weeks or three female. (f) A low incidence of malformations (renal and hepatic cysts; kidney and ovarian agenesis; agenesis of portions of brain) continues to be associated with the tumors described above. (g) Several tumor-bearing animals have been found (with Dr. Stranzinger, Pathologic Physiology Branch) to have chromosomal abnormalities (translocations, deletions). (h) Tissue cultures have been established by Dr. W. Wood, Virus Leukemia and Lymphoma Branch, NCI, from several ENU-induced tumors including nephroblastomas. Unfortunately a number of these lines were lost as the result of a contamination problem in the collaborator's laboratory. The atypical behavior of the nephroblastic cells in vitro (lack of attachment) is under investigation by Dr. Woods. Thus far attempts to re-establish tumors in the opossum by inocculation of tissue culture cells have been unsuccessful. (i) Manuscripts describing the intraoccular, nephroblastic and oral tumors are in preparation. A monograph summarizing the entire study is planned. (j) Initiation of retrospective studies to characterize the stage of development of the various target organs at the time of ENU insult have been delayed by refusal of the committee established to monitor opossum usage to approve release of necessary animals. While this decision has recently been overruled by the Director, completion of this work will depend on availability of animals and continued technical support. (k) Pilot studies to compare the effects of intraperitoneal and direct intraoccular administration of ENU with those resulting from oral administration have thus far been negative for tumor induction. The lack of tumor induction by intraperitoneal administration of ENU raises the question of the role of metabolism of the drug in relation to carcinogenesis in the opossum. Single dose intraperitoneal toxicity of ENU appears at least 20X as high as that from oral administration. The absence of intraoccular tumors in animals given ENU directly into the posterior chamber of the eye implies that the tumors thus far induced have a systemic basis and are not the result of saturation of developing occular tissue by the drug. (l) Results from a pilot study in which a total of 100 mg/kg of ENU was administered in five 20 mg/kg doses given on alternate days imply that some types of tumors, particularly the intraoccular type, can be produced in higher incidence by this approach. (m) Pilot studies with three murine oncogenic RNA viruses (with Dr. Chirigos, Virus Leukemia and Lymphoma Branch, NCI) and a murine oncogenic DNA virus (with Dr. Dawe, Laboratory of Pathology, NCI) have been negative for tumor induction. Tissues from surviving animals will be forwarded to Dr. Hellman, Virus Oncology Branch, for assay. (n) A pilot study to determine the susceptibility of developing opossums to a cell line of acantosis nigrans (with Dr. Dawe) has been negative.

PROPOSED COURSE:

(1) By administrative decision responsibility for the opossum breeding program has as of Fall 1972 been transferred to the Animal Science and Technology Branch. ASTB will henceforth have the responsibility of producing opossum young using the facility and techniques we have developed. Experimental usage of opossums is now controlled by a committee consisting of Branch Chiefs.

(2) All activities except those directly connected with the carcinogenesis program and the film have been terminated. Termination of the program has resulted from decisions by the Branch Chief, PPB, to withdraw necessary technical support from the program on completion of work in progress and from refusal of the committee appointed to monitor opossum research to approve pertinent protocols.

(3) Completion of the carcinogenesis study and the opossum film will depend upon the willingness of the NIEHS to keep commitments to maintain the necessary level of technical and fiscal support. Cost is not a factor since although ample extra-NIEHS fiscal support of both these projects has been offered. However, if technical support should be reduced to levels which are insufficient to permit work in progress to be completed, several years efforts will be destroyed.

PUBLICATIONS

Jurgelski, W., Forsythe, W., Dahl, D., Thomas, L., Moore, J., Falk, H., Kotin, P., and Vogel, F.S.: The opossum as a biomedical model: III. Breeding the opossum in captivity: Facility design. Laboratory Animal Science. (In press)

Jurgelski, W., and Porter, M.D.: The opossum as a biomedical model: IV. Breeding the opossum in captivity: Methods. Laboratory Animal Science. (In press)

Jurgelski, W., and Boyd, C.W.: The opossum as a biomedical model: II. Maintenance and laboratory methods. Laboratory Animal Science. (In press)

Serial No.: NIEHS-PP-005
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Toxicity of Sex Hormones to the Opossum (Didelphys marsupialis virginiana Kerr)"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: William Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: Pearlie M. Hudson, Clyde W. Boyd, and David Rowlands, M.D., University of Pennsylvania

COOPERATING UNITS: Pathology Department, Veterans Administration Hospital, Durham, North Carolina
Pathology Department, University of Pennsylvania Medical School, Philadelphia, Pennsylvania

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Attempts to improve reproduction performance in the female opossum through the use of serum and pituitary gonadotropins to induce estrus and estrogen and progesterone to insure receptivity to the male have resulted in a syndrome characterized by anorexia, elevated blood urea nitrogen, elevated serum potassium, gastric ulcers and death within three weeks post treatment. Preliminary work indicates that the syndrome can be reproduced with estrogen alone. The response of the opossum to estrogen is apparently unique among mammalian species and implies either a supersensitivity or an inefficient or inadequate detoxification mechanism. The objective of this study is to define the mechanism of estrogen induced injury in the opossum.

METHODS EMPLOYED: Histologic study at the light and electron microscopic levels, clinical chemistry, hypophysectomy, oophorectomy and adrenalectomy are being employed.

MAJOR FINDINGS: None. Histopathologic evaluation of tissue from the estrogen toxicity study has been delayed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Elucidation of the toxic and physiologic mechanisms of action of mammalian hormones is at an early stage. Further knowledge of the physiology and pathophysiology of the estrogens is particularly crucial in view of the large segment of the human female population currently exposed chronically to estrogen derivatives for contraceptive purposes and acutely for treatment of infertility and menstrual dysfunction. A study of the atypical response of the opossum to estrogen may give insight into both normal and abnormal estrogen metabolism and function in eutherian mammals.

PROPOSED COURSE: Terminated due to lack of support.

Serial No.: NIEHS-PP-006
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Acute and Subacute Methylmercury Intoxication in Rats"

PREVIOUS SERIAL NUMBERS: NIEHS-PP-006,007,009,015,017

PRINCIPAL INVESTIGATOR: Ronald Klein, M.D.

OTHER INVESTIGATORS: Bill C. Bullock, D.V.M., NIEHS Consultant, Bowman-Gray School of Medicine, Winston-Salem, N.C.
Martin R. Krigman, M.D., NIEHS Consultant, University of North Carolina at Chapel Hill
George Somjen, M.D., Ph.D., NIEHS Consultant, Duke University, Professor of Physiology and Pharmacology
J. Goodrich, M.D., W. Briner, Ph.D., Duke University, Division of Nuclear Medicine
Felton Hastings, Ph.D., Forestry Division, Department of Agriculture
Fred A. Talley, Animal Science and Technology Branch
Barbara E. Klein, M.D., Department of Preventive Medicine, University of North Carolina at Chapel Hill
Paul E. Brubaker, Ph.D., Environmental Protection Agency
George W. Lucier, Ph.D., Pharmacology-Toxicology Branch
Sheldon P. Herman, M.D., Resident, West L.A. Veterans Administration Hospital

COOPERATING UNITS: None

MAN YEARS

Total:	3.0
Professional:	2.0
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: To conduct clinical, electrophysiologic, metabolic, biochemical and morphologic studies in acutely or subacutely methylmercury intoxicated adult male rats with special emphasis on the central nervous system and kidney.

METHODS EMPLOYED: Adult male Charles River C-D strain rats were treated with daily subcutaneous injections of aqueous solution of methylmercury hydroxide according to the following dosage schedules:

Acute Schedule A: 10 mg Hg/kg of body weight for 1 to 10 days.
Subacute Schedule B: 2 mg Hg/kg of body weight for 1 day to 6 weeks.

Animals were observed and sacrificed from one day to 1-3/4 year after the first dose of methylmercury.

(1) Blood studies included BUN; serum creatinine; complete blood count; serum protein electrophoresis; SGOT; SGPT; serum electrolytes; total, inorganic and organic mercury levels and acetylcholinesterase levels. (2) Electrophysiologic studies included measurement of direct nerve conduction velocity, amplitudes of compound action potentials, electromyograms, twitch tensions and intraganglionic cell potentials. (3) Morphologic studies included autoradiography using Hg^{203} labeled CH_3HgOH, labeled L-lysine and routine light and electron microscopic observation of tissue fixed by in vivo perfusion with glutaraldehyde. (4) Retinal vessels were studied by trypsin digest technique and cell division was studied in lens epithelial cells.

MAJOR FINDINGS:

(1) Biochemical Changes: (a) Methylmercury was found to be a noncompetitive inhibitor of serum acetylcholinesterase activity. (b) Methylmercury led to increased hexobarbital sleeping times. (c) Methylmercury led to enhancement of DNA, RNA and protein synthesis in brain and liver and inhibition of RNA and protein synthesis in the brain (rats treated according to Schedule A). (d) Methylmercury led to increased SGOT, SGPT and inversion in albumin to globulin ratio.

(2) General Morphologic Changes Associated with Methylmercury Intoxication: (a) Spleen - early changes consisting of atrophy of Malphigian corpuscles and extramedullary hematopoiesis, increased hemosiderin pigment. (b) Cardiovascular - atherosclerotic and hypertensive changes in rats sacrificed 1-1/2 years after initial dose (Schedule B). (c) Connective Tissue - two fibrosarcomas with metastasis found 9 and 15 months after initial dose (Schedule B). (d) Eye - no histopathological changes in eye or in retinal vasculature.

(3) Renal Changes: (a) Few and mild changes in renal function were found. (b) Significant biotransformation of CH_3Hg to inorganic mercury in kidney, latter thought to cause focal necrosis of midcortical tubular epithelial cells. (c) Ultrastructural changes in renal proximal epithelial cells consisted of swollen mitochondria. (d) Late changes (Schedule B) revealed ongoing process of tubular epithelial cell hydropic degeneration and necrosis, regeneration and areas of interstitial nephritis distributed in both proximal and distal tubules.

(4) Central Nervous System: (a) Clinical - Reproducible clinical changes consisted of weight-loss followed by decreased activity, ataxia and hindlimb crossing phenomenon. Ataxic rats surviving over 1-3/4 years after initial dose show no reversibility of neurological signs. (b) Electrophysiologic data revealed that during the course of CH_3Hg intoxication, the compound action potential of dorsal roots showed gradual retardation of the conduction velocity, elevation of the extracellular threshold and a reduction of the amplitude and "area" of the compound spike; intracellular recordings of spinal ganglion cells revealed an abnormal pattern. (c) Morphologic - Autoradiograms revealed

localization of ^{203}Hg labeled CH_3HgOH in gray matter primarily in neurons and satellite cells and decreased uptake of labeled L-lysine in dorsal root ganglion cells which suggests impairment of protein synthesis in these cells. Light and electron microscopy revealed necrosis in putamen, internal granular cell layer of cerebellum and a sensory peripheral neuropathy beginning in the spinal ganglion neuron cell bodies producing a Wallerian-like degeneration in nerve fibers (preceding clinical and physiological evidence of the disease by 1-2 weeks). In addition, a new technique has been devised whereby intracellular injection of specific dyes after recording is finished allowed us to indentify, by light microscopy, the specific cell studied.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We combined a multidisciplinary approach to study and correlate the clinical, electrophysiologic, biochemical and pathological sequence of events during and following acute and subacute methylmercury intoxication in rats. Significant contributions were made to understanding of the fundamental pathogenesis of central nervous system toxicity in rats. We presented the first detailed electron microscopic study of CH_3Hg poisoned ganglion cells, and thus firmly established the direct toxicity of CH_3Hg on these cells. It is hoped that this knowledge may lead to improved diagnostic and therapeutic approaches to CH_3Hg poisoning in humans. In a basic sense, the interesting and unusual effects of CH_3Hg on neuron membrane dynamics and structure may lead to a fundamental breakthrough in neuronal membrane excitability theory.

We have also identified CH_3Hg as more than just a neurotoxic agent. Functional and/or structural alterations in CH_3Hg intoxication in the liver (impaired detoxification mechanism, alterations in protein synthesis), kidney (tubular necrosis leading to chronic nephritis), spleen (decrease in small lymphocytes) and blood (decrease in serum albumin/globulin ratio, decreased serum acetylcholinesterase levels) have been described in detail for the first time. These findings reveal that CH_3Hg effects many target organs and that these effects must be further studied and the results should be useful in establishing maximum allowable concentrations of CH_3Hg in the environment.

PROPOSED COURSE: Studies are scheduled for completion by June 1973.

PUBLICATIONS

Klein, R., Herman, S.P., Brubaker, P.E., Lucier, G.W., and Krigman, M.R.: A model of acute methylmercury intoxication in rats. Arch. of Path. 93: 408-418, 1972.

Lucier, G.W., McDaniel, O., Brubaker, P.E., and Klein, R.: Effects of methylmercury hydroxide on rat liver microsomal enzymes. Chem. Biol. Interactions 4: 265-280, 1972.

Lucier, G.W., Klein, R., Matthews, H.B., and McDaniel, O.S.: Increased degradation of rat liver CO-binding particles by methylmercury hydroxide. Life Sciences, Part II, 11: 597-604, 1972.

Lucier, G.W., McDaniel, O.S., Williams, C., and Klein, R.: Effects of chlordane and methylmercury on the metabolism of carbaryl and carbofuran in rats.

Pesticide Biochem. and Physiol. 2: 244-255, 1972.

Klein, R., Brubaker, P., Herman, S., and Lucier, G: Liver and kidney changes during the latent phase of acute methylmercury intoxication in rats. Proceedings of VII Annual Meeting of Clinical Society and Commissioned Officers Association, New York, May 30 to June 2, 1972.

Krigman, M.R., Herman, S., Klein, R., Briner, W.H., and Somjen, G.G.: Experimental Minamata disease: pathogenesis as related to the distribution of methylmercury in the rat nervous system. Annual Meeting of Amer. Assoc. of Neuropathology, Chicago, 1972.

Klein, R., Herman, S.P., and Bullock, W.: The renal pathology of methylmercury intoxication in the rat. Proceedings of the Fifth International Congress of Pharmacology, San Francisco, July, 1972.

Herman, S., Somjen, G., Klein, R., Goodrich, J., and Brubaker, P.: Distribution of methylmercury^{203} Hg in the rat nervous system. Proceedings of the Fifth International Congress of Pharmacology, San Francisco, July, 1972.

Mushak, P., Zarnegar, P., Fisher, G.B., Lucier, G.W., and Klein, R.: Levels of distribution of inorganic and methylmercury in tissues of rats and mice administered multiple doses of methylmercury. Annual Meeting of Southeastern Chemical Society, Atlanta, 1972.

Herman, S.P., Klein, R., Talley, F.A., and Krigman, M.R.: An ultrastructural study of methylmercury induced primary sensory neuropathy in the rat. Laboratory Investigation 28: 104-118, 1973.

Brubaker, P.E., Klein, R., Herman, S.P., Lucier, G.W., Alexander, L., Long, M.: DNA, RNA and protein synthesis in brain, liver and kidneys of asymptomatic methylmercury treated rats. Exper. and Mol. Path. (In Press).

Lucier, G.W., Matthews, H.B., Brubaker, P.E., Klein, R., McDaniel, O.S., and Mushak, P.: Effects of methylmercury on microsomal mixed function oxidase components of rodents. J. Mol. Pharm. (In Press).

Somjen, G.G., Herman, S.P., Klein, R., and Krigman, M.R.: Electrophysiology of methylmercury poisoning. J. Pharm. Exper. Therap. (In Press).

Klein, R., Herman, S.P., Bullock, B.C., and Talley, F.A.: Early functional and pathological changes in rat kidney during methylmercury intoxication. Arch. Path. (In Press).

Serial No.: NIEHS-PP-026
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Passage of Environmental Chemicals Across the Placenta and Concentrations Achieved in Fetal and Maternal Tissues"

PREVIOUS SERIAL NUMBER: NIEHS-PP-026,027

PRINCIPAL INVESTIGATOR: Herbert S. Posner, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNIT: Animal Science and Technology Branch

MAN YEARS

Total:	1.9
Professional:	0.9
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the passage of toxic environmental chemicals of several structural types across the placenta and comparative accumulations in fetal and maternal tissues, (2) to determine if pre- or co-administration of selected other compounds can affect the rate of passage or the amounts that accumulate and (3) to determine if a correlation exists between one or more of these parameters and toxicity to the conceptus.

METHODS EMPLOYED: Guinea pigs are first anaesthetized with sodium pentobarbital administered intraperitoneally and then maintained by inhalation of methoxyflurane. In a few experiments, one of the placentas was prepared for in situ perfusion. ^{14}C-Paraquat, ^{14}C-dieldrin and chemicals that might alter placental transfer or organ accumulation are administered to the mother intravenously. Appearance of radioactivity on the fetal side of the placenta in the perfusate, and/or the concentrations in analogous maternal and fetal organs is determined. In this experimental arrangement, accumulation in fetal and maternal tissues is a function of transport both to and from the fetal compartment. Attempts will be made later to compare the rates of transfer across the placenta in the two directions.

MAJOR FINDINGS: In the ten experiments, ^{14}C-paraquat was administered to the mother on a day between 34 and 64 of gestation; in another ten it was administered immediately, 10, 20 or 30 minutes after administration of norchlorcyclizine on a day between 36 and 64. Concentrations of radioactivity achieved in

the fetal tissues were adjusted to constant and average maternal plasma concentration for experiments in which the plasma concentration was not held experimentally constant by intermittent infusion. Under these conditions, the radioactivity in the fetal kidneys, heart, lungs, liver and brain linearly increased by 2-3 times between 25 min. and the maximum time of assay, 75-90 min., at each stage of gestation tested. The relative concentrations attained in the fetal tissues decreased in the order from kidneys to brain above. No difference was seen between the paraquat and paraquat + nochlorcyclizine experiments. Presently it is unknown how long the paraquat concentration in the fetal tissues would continue to increase. In an earlier work in the rat, the radioactivity in the neonatal lung increased for two days after birth following administration of ^{14}C-paraquat on day 20 or 21 of gestation.

In seven experiments, ^{14}C-dieldrin was administered on a day between 33 and 64; in another two it was administered immediately following the norchlorcyclizine on day 46 or 62. Concentration of radioactivity was again adjusted to the average maternal plasma concentration and actually, the maternal plasma concentration was constant up to 75 min. after an initial unpreventable phase of rapid decline. After the initial accumulation, radioactivity in the fetal liver, heart, brain, lungs, and kidneys was essentially constant between 25 and 75 minutes. The relative concentrations attained in the fetal tissues decreased in the order from liver to kidneys above. While the fetal liver accumulated more at later stages of gestation, stage-dependent change of accumulation was not seen in the other organs. No difference was observed between the dieldrin and dieldrin + norchlorcyclizine experiments. Radioactivity was found in the fetal bile and thus enterohepatic circulation might be a factor in the fate of dieldrin and/or its metabolites in the fetus.

When, at the end of gestation, the organs of highest concentration were compared, it was found that 10-15 times the concentration of radioactivity appeared in the fetal liver after dieldrin as occurred in the fetal kidneys after paraquat. However, fetal liver reached its maximum amount following dieldrin after the initial 25 min., while radioactivity in the fetal kidneys after paraquat was still increasing linearly.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Transport across the placenta is known to be a function of the physical characteristics of the chemicals studied: molecular weight, ionic charge and lipid-like solubility. Accumulation in fetal tissues is also a function of biological parameters: blood flow at several loci, the state of maturation of fetal organs, binding to macromolecules and to a variety of other changes in the characteristics of maternal and placental compartments with the progression of gestation. This wide-ranging state suggests (1) that the prediction of rates and levels of accumulation in the fetus can be made, in most cases, only with great variation and (2) that special situations might arise frequently. It is the purpose of the project, first to learn about the pattern of accumulation of environmentally important chemicals by choosing several from classes representing different physical characteristics. A second aspect is to determine which of several types of secondary chemical administrations might alter the placental transfer or accumulations of the environmental compound in the fetus. Thus, paraquat, a quaternary ammonium herbicide which accumulates in animal and human lungs and which has been the cause of over 124 human deaths

in children and adults (Lancet, Nov. 6, 1971, 1018-19; JAMA, Feb. 5, 1973, 692) and dieldrin, a pesticide which is a neutral, chlorinated hydrocarbon persistent in the environment and which can produce neurologic symptoms in humans, have been chosen first. Norchlorcyclizine was selected as the initial secondary agent to be tested. The latter is a metabolite of several drugs known to be teratogenic in animals and is a compound which the investigator has studied over the past few years.

PROPOSED COURSE: Efforts to evaluate the placental transfer of environmental agents and to determine the factors which affect relative fetal and maternal concentrations of chemicals in the blood and at receptor sites will be continued.

Serial No.: NIEHS-PP-028
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Induction of Resistance to Pesticides in Cultured Lymphoma Cells

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Judson W. Spalding, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	1.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To study the toxicity of pesticides and other xenobiotics in mammalian cell lines in culture and to compare in normal and resistant cell lines the mechanism by which toxicity and resistance are expressed using appropriate biochemical and metabolic parameters.

METHODS EMPLOYED: Mouse L5178Y lymphoma sublines which are resistant to specific organochlorine pesticides and related xenobiotics have been isolated. The effect of selected organochlorine compounds, DDT and 4-chlorobiphenyl, on transport into cells and subsequent incorporation of appropriate precursors into DNA, RNA, protein and phospholipids has been studied and compared in normal and resistant cell lines.

MAJOR FINDINGS:

(1) The transport into cells and the subsequent incorporation of selected precursors into various macromolecular species were compared in normal and DDT resistant cell lines. The most significant differences between normal and DDT resistant cells were found in the rates of membrane transport and uptake of myo-inositol and the pyrimidine-riboside precursors. Resistant cells were able to incorporate myo-inositol at a rate 30-50% higher than control cells. However, DDT was equally inhibitory to inositol uptake in both the normal and resistant cell lines. Is is not known at this time whether the inhibition of inositol uptake by DDT is the means by which the toxicity of DDT to these cells is

expressed. But the resistant cell line is apparently able to overcome this specific effect of DDT by incorporating inositol from the medium at a faster rate than normal cells so that the cell is not deficient in inositol which is an essential nutrient for this cell line. Conversely, DDT resistant cells were observed to incorporate thymidine and uridine into whole cells at only 40-50% of the rate of normal cells. This phenomenon seemed to be specific for the pyrimidine ribosides as there was no difference in the rates of whole cell uptake of the purine ribosides, guanine and adenine.

(2) Toxicity of 4-chlorobiphenyl: The toxicity of the chlorinated biphenyls was investigated in mouse L5178Y lymphoma cells. Of several monochloro and dichloro-biphenyl compounds, and 4-chlorobiphenyl was found to be the most toxic. The ID_{50} concentration was 6.0 $\pm$ .1 µgm/ml. In the studies carried out thus far, the most striking effect of 4-chlorobiphenyl appears to be on phospholipid metabolism, specifically with respect to phosphatidylethanolamine and phosphatidylcholine synthesis. The incorporation of ethanolamine into the phosphatide is inhibited. Conversely, the incorporation of choline into phosphatidylcholine is stimulated up to 3 fold, though the initial rate of choline transport into whole cells is similar in treated and untreated cells. How these observations are related to the toxicity of 4-chlorobiphenyl is unknown at this time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mode of toxicity exhibited by the organochlorine pesticides and other related xenobiotics is unknown at this time. Tissue culture cell systems provide useful models whereby the molecular mechanism of toxicity expressed by these compounds may be investigated. The resistance exhibited by the DDT-resistant cell line depends on a different mechanism than that found in the microsomal enzyme system of liver, lung and other tissues. Thus these cells, which do not have the usual microsomal detoxification system, are useful for studying alternative mechanisms of resistance. These studies will also lead to the understanding of how DDT and other highly water-insoluble and persistant organochlorine compounds effect their toxicity in biological systems.

The results of the investigations described above suggest that these compounds express their toxicity by altering the rate of transport of essential small precursor molecules into cells (tissues). In addition, they alter the metabolism of phospholipids and thus can effect the integrity of the various membrane structures of cells (tissues).

PROPOSED COURSE: These studies on phospholipid metabolism will be continued on a limited basis.

PUBLICATIONS

Spalding, J.W., Ford, E., Lane, D., and Blois, M.: The character of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane resistance in mouse L5178Y lymphoma cells. Biochem. Pharmac. 20: 3185-3196, 1971.

Serial No.: NIEHS-PP-032
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis"

PREVIOUS SERIAL NUMBER: None

PRINICPAL INVESTIGATORS: Insu P. Lee, Ph.D., and Robert L. Dixon, Ph.D.

OTHER INVESTIGATORS: Rong-Sen Shen, Ph.D., Katsuhiko Okumura, Ph.D., and Judson W. Spalding, Ph.D.

COOPERATING UNIT: Hazleton Laboratories, Inc.

MAN YEARS

Total:	3.3
Professional:	2.5
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate biochemical and toxic effects of environmental agents, including drugs on mouse spermatogenic cells using both velocity sedimentation cell separation and serial mating techniques, (2) to define enzyme development during spermatogenesis and attempt to develop useful "marker" enzymes to indicate normal cellular differentiation and toxic effects and (3) to investigate the nature of blood-testicular barrier and to elucidate the importance of this permeability barrier to the penetration of various environmental chemicals (mutagens, carcinogens and drugs) to spermatogenic cells.

METHODS EMPLOYED:

In vivo: Serial mating of male mice was used to assess the biological functionality of sperm cells and to produce fertility patterns which have been shown to be inversely related in time to the phase of spermatogenesis damaged by the test chemical.

In vitro: The modified cell separation technique of Lam, Furrer and Bruce is being used. This allows one to separate various spermatogenic cell types by velocity sedimentation in an albumin gradient. Attempts are being made to culture embryonic and postnatal germinal tissues with special emphasis on the germinal ridge and spermatogonia. In addition, routine pharmacologic,

biochemical and morphologic procedures are used.

MAJOR FINDINGS:

Spermatogenesis:

a. Cadmium. The velocity sedimentation cell separation technique was used to identify the spermatogenic cell types which incorporate cadmium and zinc, and to study the interaction of these two metals. ^{109}Cadmium and ^{65}zinc were used to monitor the cellular incorporation. Following intraperitoneal administration of either cadmium or zinc, peak radioactivity was present in the spermatogenic cell fraction which sediments at 2.2 m/hr (late elongated spermatids). The incorporation of these two metals into the late elongated spermatids appeared to be competitive. Cadmium chloride (1 mg/kg), administered i.p., immediately inhibited thymidine uptake into spermatogonial cells but cadmium failed to affect the incorporation of uridine and L-leucine into early and late elongated spermatids, respectively. In vivo serial mating fertility studies indicated that cadmium affected all spermatogenic cell types, with the possible exception of mature spermatozoa, and significantly decreased fertility. No dominant lethal effect was found during a 55-day period following a single cadmium injection. Pretreatment of mice with $ZnCl_2$ (1 mg/kg) prior to cadmium treatment completely blocked the biochemical and functional effect of cadmium on spermiogenic cells but not on spermatogonial cells. Therefore, the primary action of cadmium seems to be both an effect on zinc utilization by spermiogenic cells and an inhibition of DNA synthesis by spermatogonial cells.

b. Mercury. Testicular half lifes of methylmercury after administration of 1 mg/kg intraperitoneally were 8 days for the fast component and 14.5 days for the slow component. The in vitro effects of methylmercury and mercuric chloride on the uptake of ^{3}H-thymidine by spermatogonia, ^{3}H-uridine by early spermatids and ^{3}H-L-leucine by late elongated spermatids indicated that methylmercury reduced thymidine incorporation to 60.2%, uridine incorporation to 90% and L-leucine to 87% at 10^{-6}M. Inorganic mercury has no effect at this concentration. The in vivo effects of methylmercury on the uptake of ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-L-leucine into the respective spermatogenic cell types on day 1 after a single i.p. dose of 1 mg/kg were studied. Significant inhibition of uptake of thymidine and L-leucine, but no inhibition of uridine uptake was observed. Fertility profiles obtained from serial mating studies indicated that the primary effect of methylmercury was on the spermatogonial cell series, premeiotic spermatocytes and early elongated spermatids. Complete infertility was observed on day 42 and recovery of fertility was seen after 60 days which appears to correlate well with inhibition of thymidine uptake determined in vitro. No significant effects were seen with mercuric chloride at the same dose.

Testicular Enzymes. An activity profile of 11 different mouse testicular enzymes as a function of age from birth to 10 weeks old was determined. These enzymes were lactic dehydrogenase, sorbitol dehydrogenase, glucose-6-phosphate dehydrogenase, glycerphosphate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, glucose-3-phosphate dehydrogenase, hyaluronidase, carboxy peptidase A, chymotrypsin-like and trypsin-like enzymes. The results of

preliminary experiments showed that most of the enzyme activities sharply increase about the 25th day after birth. The mechanism of this sharp rise in enzyme activities is not known at the present. Studies will be continued to search for enzymes or isozymes which are specific for a particular spermatogenic cell type.

Blood-Testicular Barrier. Studies to assess the permeability of the blood-testicular barrier have just been initiated. Attempts will be made to define this "barrier" from a physiologic and/or morphologic standpoint, and to compare its patency to that of other biologic membrane systems such as the placental and blood-brain barrier. Experimental techniques which have been used previously to study these other barriers are being employed. The penetration of environmental toxicants to the seminiferous tubular cells will be analyzed to determine the effect of the barrier during dominant lethal testing.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies of the toxic effects of environmental agents on reproduction in the past have centered largely on teratogenicity. Few investigations have been concerned with the effect of chemicals upon fertility. These studies have demonstrated that the male reproductive system is vulnerable to the toxic effects of a variety of chemical agents in the environment. Serial mating tests allow one to construct fertility profiles suggesting biochemical alterations which are then correlated with biochemical effects associated with specific cell types isolated by velocity sedimentation. Studies of enzyme patterns associated with aging and cellular differentiation will not only help to explain toxic effects, but should also help to reveal some of the basic mechanisms associated with gametogenesis and male fertility.

PROPOSED COURSE: Studies will be continued to assess the toxic effect of environmental agents on spermatogenesis and to investigate further "testicular marker enzymes", the blood-testicular barrier and spermatogenic cell cultures. Attempts will be made to define the interrelationship of these factors from a basic and applied standpoint.

PUBLICATION

Lee, I.P., and Dixon, R.L.: Effects of mercury on spermatogenesis studied by velocity sedimentation cell separation and serial mating. Appl. Pharmacol. and Toxicol., p. 51, 12th Annual Meeting, New York, N.Y., March 18-22, 1973.

Serial No.: NIEHS-PP-033
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effect of Foreign Chemicals on Oogenesis and Ovarian Function in Mammals"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: John A. McLachlan, Ph.D., and Robert L. Dixon, Ph.D.

OTHER INVESTIGATOR: Judson W. Spalding, Ph.D.

COOPERATING UNIT. Hazelton Laboratories, Inc.

MAN YEARS

Total:	2.7
Professional:	1.7
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To evaluate the effects of prenatal exposure to foreign chemicals on the subsequent reproductive capacity of the offspring, (2) to investigate the mechanisms involved in the production of subfertility in adults as a result of their *in utero* exposure to foreign chemicals, (3) to assess the transplacental carcinogenic potential of these compounds, (4) to study the distribution of compounds between blood plasma and adult or fetal ovarian compartments, (5) to compare the DNA synthetic period during oogenesis in various species, and (6) to develop an embryonic mouse organ culture system for the study of oogenesis *in vitro*.

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is total reproductive capacity as determined by repetitive forced breeding techniques. These techniques are coupled with the determination of ovarian periodicity by daily vaginal smears, superovulatory challenges with exogenous gonadotropins. Histopathologic evaluation of the ovary including quantitative determinations of the various follicular classes to evaluate the functional status of the female gonad are also performed. Routine biochemical, physiologic and morphologic procedures are also used which include electrophoresis, histochemistry, autoradiography and microdissection.

MAJOR FINDINGS: Initial experiments to evaluate the effects of foreign chemicals on oogenesis were done using two model compounds known to effect the DNA of mammalian cells. Procarbazine (NSC-77213) or cyclophosphamide (NSC-26271) were

given in subteratogenic doses to pregnant mice on either the 10th, 12th or 17th day of gestation; these times correspond, respectively, to the periods before, during and after maximal oocyte DNA synthesis. Prenatal exposure to procarbazine on the 12th gestational day produced female offspring which delivered less than half as many young as the corresponding control mice during the last six months of a one-year forced breeding study. During the same time period all cyclophosphamide treatment groups had their fertility reduced by 30%. In addition, both compounds apparently produced tumors in mice exposed to them in utero.

These studies were expanded to include compounds of more widespread environmental importance such as DDT, diethylstilbesterol and 2,3,7,8-tetrachlorodibenzo-p-dioxin. For example, one year after prenatal exposure to DDT (1 mg/kg of maternal body weight) female mice cumulatively delivered 52% fewer young than did control offspring.

This apparent subfertility could not be explained by initial differences in estrous cycles, ovarian morphology, responsiveness to gonadotropins or mating behavior. However, efforts are being made to utilize biochemical methods to predict or assess these ovotoxic effects of foreign chemicals. The changes in enzyme activity levels and isoenzyme patterns which accompany ovarian development are being evaluated. The initial focus is on enzyme such as carbonic anhydrase, asparagine synthetase, acid and alkaline phosphatases and non-specific esterases. In addition, experiments are currently underway to generate an in vitro system to study mammalian oogenesis under controlled conditions. Pilot studies have been done to isolate, explant and culture in chemically-defined media the gonadal primordia of 11-day old mouse embryos. Also of interest are comparative studies related to oogenesis in the opossum.

In order to understand the effects of foreign chemicals on oogenesis, it is important to determine the relationship between the ovotoxicity of a compound and the degree of its penetration into the fetal and adult ovary. To this end, experiments were initiated to study the distribution of some radioactively-labeled compounds (diethylstilbesterol, DDT, cyclophosphamide and procarbazine) between blood plasma, follicular fluid and the oocyte. Attempts will be made to correlate the accumulation of a foreign compound in ovarian follicular fluid and/or oocyte with the chemical's effects on ovarian function.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such in utero drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic damage to the developing oocyte, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PROPOSED COURSE: The studies dealing with the total reproductive capacity of mice exposed to foreign chemicals in utero will be continued and expanded to

include a wider range of environmental chemicals such as heavy metals. Emphasis will be placed on finding ovarian "marker" proteins which may be useful in evaluating subtle ovotoxicity which is manifested as an impairment in ovarian function without concomitant gross morphological alterations. The further improvement of the mouse embryonic gonad organ culture system will provide information on basic processes of oogenesis and, in addition, may serve as a screen for the ovotoxic potential of environmental compounds.

PUBLICATIONS

McLachlan, J.A., and Dixon, R.L: Reduced fertility in female mice exposed prenatally to procarbazine. Proceedings of the Federation of American Society of Experimental Biologists Meeting, Atlantic City, April, 1973.

McLachlan, J.A., and Dixon, R.L.: Reduced fertility in the offspring of mice treated with DDT during gestation. Abstracts of Joint Meeting of Professional Associations of the U. S. Public Health Service, Phoenix, May, 1973.

Serial No.: NIEHS-PP-034
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "In Vitro Culturing of Mammalian Gonadal Tissues"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Judson W. Spalding, Ph.D., Insu P. Lee, Ph.D., and John A. McLachlan, Ph.D.

OTHER INVESTIGATOR: Robert L. Dixon, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop methods and techniques for establishing in vitro a viable tissue or organ culture system of mammalian gonads for the purpose of investigating the mechanism by which chemical agents affect oogenesis and spermatogenesis.

METHODS EMPLOYED: This project is in its initial stages and methods are being developed. The emphasis at this time is on culturing techniques. Mammalian gonadal tissue from mice during various stages of prenatal and postnatal development is excised and maintained in a defined culture medium. The optimal conditions for maintaining these tissues in culture are being investigated. Methods for following the growth and differentiation of these tissues by morphological, histochemical and biochemical parameters are also being developed.

MAJOR FINDINGS: This is a project initiated in mid-year, and no major findings can be reported at this time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Various environmental agents including drugs are known to affect reproduction and development. The specific molecular effect of these agents on germ cells (oocytes and spermatocytes) during the early stages of development are relatively unknown. It can be expected that defective germ cells may result in offspring which are sterile or have a wide range of inborn genetic deficiencies.

It would be very beneficial to have an *in vitro* model of embryonic and postnatal germ cell differentiation. *In vitro* studies could then be correlated with ongoing studies of the *in vivo* effects of chemicals on gametogenesis and fertility. Hopefully, a fast *in vitro* test system can be developed to predict *in vivo* effects of chemicals. In addition, this *in vitro* system would provide an opportunity to further study mechanisms which regulate differentiation and maturation.

PROPOSED COURSE: These studies will be expanded in the coming year with an increased effort to culture embryonic gonadal tissues as well as postnatal ovarian tissue and spermatogenic cells.

Serial No.: NIEHS-PP-035
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Role of Superoxide Dismutase in Oxygen Tolerance and Oxygen Toxicity"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: James D. Crapo, M.D.

OTHER INVESTIGATOR: Peter E. Voytek, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.3
Professional:	1.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To study the relationship between the level of superoxide dismutase (SOD) present in lung tissue and the ability of the animal to survive in pure oxygen or when given lethal doses of other pulmonary oxidants and (2) to determine the developmental characteristics of the enzyme SOD and to relate these to the changing ability of a developing animal to tolerate inhaled oxidants.

METHODS EMPLOYED: The aerotoxicology facilities at the Institute are used to expose test animals to various concentrations of oxygen. Routine biochemical and physiologic testing procedures are also used.

MAJOR FINDINGS:

(1) Characteristics of Rat SOD. It has been shown that all organs in the rat contain SOD and that the electrophoretic mobility of the enzyme is identical in all organs. Rat liver SOD has been isolated and purified and specific activity determined.

(2) Pulmonary SOD and Oxygen Toxicity. It has been shown that rat lung SOD activity is elevated to 150% of control levels during a continuous 7-day exposure to 85% oxygen (sublethal concentration). These animals with increased SOD levels have been shown to survive in 100% oxygen while all control animals die under these experimental conditions. The time course of the increase in pulmonary SOD

activity has been shown to correspond to the time course of the development of oxygen tolerance in the rat. Following a 7-day exposure to 85% oxygen, the time course of the return of SOD activity to control levels has also been shown to correlate with the duration of tolerance. White blood cells, red blood cells, platelets and plasma have been studied and shown not to contribute to the increased activity of SOD in lungs of rats exposed to 85% oxygen.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The single electron reductions of oxygen, producing an extremely toxic free radical known as superoxide, have recently been shown to be a biologically important reaction. The enzyme SOD dismutes this free radical directly to O_2 and H_2O_2. It has been postulated that the presence of this enzyme is protective against the toxic effects of oxygen and that it is necessary for survival in an oxygen atmosphere. Oxygen is one of the most frequently used forms of medical therapy, and artificial atmospheres of oxygen are used in both industry and in medicine. All of the initial toxic effects of oxygen are manifest in the lung and deaths due to pulmonary oxygen toxicity are frequently reported. The current studies represent one of the first attempts to propose a biochemical mechanism for pulmonary oxygen toxicity.

A large number of other environmental toxicants including ozone, NO_2 and paraquat may produce part of their toxic effects by way of the superoxide free radical, and the current studies with SOD and oxygen may have broad applicability with regard to environmental agents.

The developmental aspects of SOD should contribute to our understanding of the differing and changing abilities of neonatal animals to respond to pulmonary oxidants. Abnormalities of SOD during development might be related to the development of hyaline membrane disease.

PROPOSED COURSE: (1) A study of developmental characteristics of pulmonary, blood and liver SOD from fetal to adult stages is proposed. (2) Further attempts will be made to compare the induction of increased activity of SOD in the lung with the development of oxygen tolerance. Other species will be studied and the effects of injected SOD will be evaluated. (3) Other pulmonary oxidants will be evaluated for the development of cross tolerance with oxygen and for their ability to increase SOD activity in the lung.

PUBLICATION

Tierney, D. and Crapo, J.: Superoxide dismutase and oxygen toxicity. Abstracts of Western Federation of Medicine, 1973.

Serial No.: NIEHS-PP-036
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Morphologic Effects of Heavy Metals on Mammalian Tissues"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Bruce A. Fowler, Ph.D.

OTHER INVESTIGATORS: Robert A. Goyer, M.D., Department of Pathology, University of North Carolina at Chapel Hill
Helena J. Jones, Ph.D., Officer of the Director
Kathryn R. Mahaffey, Ph.D., F.D.A., Washington, D.C.
George W. Lucier, Ph.D., Pharmacology-Toxicology Branch
Margaret D. Folsom, Ph.D., Animal Science and Technology Branch
Shang W. Hwang, Ph.D., Pharmacology-Toxicology Branch
Martin R. Krigman, M.D., Department of Pathology, University of North Carolina at Chapel Hill
Paul Mushak, Ph.D., Department of Pathology, University of North Carolina at Chapel Hill

COOPERATING UNITS: Department of Pathology, University of North Carolina at Chapel Hill
F.D.A., Washington, D.C.
Pharmacology-Toxicology Branch
Animal Science and Technology Branch

MAN YEARS

Total:	3.2
Professional:	2.2
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To define the ultrastructural effects of heavy metals and other environmental toxicants on mammalian tissues and (2) to correlate these observations with tissue levels and biochemical alterations in blood and viscera.

METHODS EMPLOYED: Routine chemical, histologic and electron microscopic methods are used. Special emphasis is placed on the determination of tissue levels of heavy metals and subcellular morphologic changes.

MAJOR FINDINGS:

(1) Arsenic. Exposure of rats to arsenic in the drinking water at levels of 3, 30, 45, 85 and 120 parts per million (ppm) for 6 or 12 weeks was found to produce subcellular alterations in renal proximal tubule cells. Proliferation of smooth endoplasmic reticulum (SER) and enlarged microbodies (peroxisomes) were noted at all dose levels. At doses of 45 ppm and above, swollen mitochondria and necrotic cells were observed. Biochemical studies disclosed increased catalase activity associated with 3 ppm arsenic and decreased mitochondrial respiration at 85 and 120 ppm.

(2) Cadmium. The effects of low and normal dietary calcium levels on rats given cadmium in the drinking water at dose levels of .2, 2, 20 and 200 ppm were studied. Testicular necrosis, parathyroid hyperplasia, vascular damage and proliferation of SER in kidney and liver cells were observed in animals receiving either diet. These changes were more prominent in animals given the two higher doses of cadmium. Rats receiving the normal calcium diet concentrated more cadmium in the kidneys than in the liver while those receiving the low calcium diet had roughly equal concentrations of cadmium in the liver and kidneys. Growth curves indicated that animals given the low calcium diet did not gain weight as fast as those on the normal calcium diet. Increased BUN levels and altered Ca/PO_4 ratios were also noted in rats given cadmium on either diet.

(3) Methylmercury. Previous studies have indicated decreased morphological and biochemical toxicity associated with methylmercury when animals are simultaneously exposed to inducers of microsomal enzyme activity. The present study was undertaken to evaluate the effects of phenobarbital on the morphological and biochemical aspects of methylmercury toxicity and to correlate these findings with tissue levels of methyl and inorganic mercury. Decreased morphological alteration of the kidneys and nervous system was observed in rats ingesting methylmercury at the dose of 5 ppm for four weeks but receiving injections of phenobarbital at a dose of 75 mg/kg. Liver microsomal enzyme activity which was depressed by methylmercury was stimulated by phenobarbital. These findings were correlated with decreased renal levels of organic methylmercury but not inorganic mercury noted in brains of animals given phenobarbital. An overall stimulation of methylmercury detoxification by phenobarbital is indicated.

(4) Dioxin. Combined ultrastructural and biochemical studies were carried out on livers of rats receiving a single injection of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) at doses of 5 µg/kg and 25 µg/kg. Profound changes in both the rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER) were observed by electron microscopy in treated animals. Proliferation of SER was most marked around the periphery of parenchymal cells, particularly in areas adjacent to bile canaliculi. A massive increase in RER was seen throughout these cells. The above changes were correlated with an increase in microsomal enzyme activity and biochemical estimates of RER levels.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to delineate ultrastructural criteria for evaluating the toxicity of environmental agents administered at low levels. These findings

are associated with specific biochemical events and tissue concentrations of these agents. Because the tissue requirements for electron microscopy are small, it might eventually be possible to evaluate the toxicity of environmental agents, e.g., heavy metals, through the use of biopsy procedures.

PROPOSED COURSE: The effects of heavy metals on cellular ultrastructure will be continued. In addition, the influence of one heavy metal on the toxicity of another will also be investigated. Further studies are anticipated in order to compare observed cellular alterations of heavy metals deposited in biologic tissue with those seen when the metals are given in drinking water or added to food.

PUBLICATIONS

Fowler, B. A., Lucier, G. W., Folsom, M. D., Brown, H., and Mushak, P.: Phenobarbital protection against methylmercury nephrotoxicity: An ultrastructural study. Abstracts of Conference on Heavy Metals in the Environment, Research Triangle Park, N. C., March, 1973.

Fowler, B. A., Lucier, G. W., Brown, H., and McDaniel, O.: Ultrastructural changes in rat liver cells following a single injection of TCDD. Environ. Health Perspectives, Experimental Issue No. 5, August, 1973. (In Press)

Fowler, B. A., Brown, H. W., Lucier, G. W., and Mushak, P.: Ultrastructural evaluation of protection against methylmercury nephrotoxicity by sodium phenobarbital. Proceedings of the Federation of American Society of Experimental Biologists Meeting, Atlantic City, April, 1973.

Serial No.: NIEHS-PP-037
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of Thymidine Kinase Enzymes in Independently Derived Mutant Lines of L5178Y Mouse Lymphoma Cells"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Peter E. Voytek, Ph.D.

OTHER INVESTIGATOR: Donald Clive, Ph.D., Mutagenesis Branch

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	1.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate whether thymidine kinase activity in the various thymidine kinase mutant cells is caused by mutations in control or structural genes; and (2) if structural changes are evident, to carry out studies of the physical and kinetic parameters of the enzymes.

METHODS EMPLOYED: Production and isolation of various mutant stock cells are being obtained as described in Individual Project Reports, Serial No. NIEHS-M-004 and NIEHS-M-006, July 1, 1971 through June 30, 1972. Purification techniques are presently being carried out to enable characterization of the different mutated thymidine kinases. Electrophoretic techniques, density gradient centrifugation and examination of kinetic parameters will be used to investigate enzymatic differences. Thymidine kinase from the original lymphoma cell line which contains the diploid locus is being investigated first to assess the characteristics of these two (possibly identical) gene products.

MAJOR FINDINGS: Initial attempts to purify thymidine kinase were complicated because the enzyme was not extracted in the supernatant fluid from sonnicated cell fractions. Other techniques such as freezing and thawing the cells, Triton X treatment, butanol extraction and sodium-dodecyl sulfate treatment were unsuccessful in solubilizing the enzyme. However, sonnication in 3M LiCl released 50-80% of thymidine kinase into the supernatant fluid. $MgCl_2$, EDTA and β-mercaptoethanol were included in the extraction buffer for

stabilization of the enzyme. The enzyme preparation can be stored without loss of activity at 4^{o} for at least a month. Storage at -70^{o} caused considerable loss of enzymic activity within a day. Further purification has been achieved by electrophoresing the LiCl preparation of the enzyme in a sucrose gradient without significant loss of activity. The preparation from this step represents a 5 fold purification and is stable at 4^{o} over several weeks. Hopefully, this purification protocol can be used for other mutant thymidine kinases so that preliminary studies comparing kinetic and physical parameters can be made.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent studies have shown that the thymidine kinase locus in L5178Y mouse lymphoma cells grown in tissue culture suspension may be used as a mutational assay system for evaluating the severity of physical and chemical mutagenic agents. Thymidine kinase deficient mutants can be isolated and later reverted to yield thymidine kinase competent mutants (see Serial No. NIEHS-M-004 and NIEHS-M-006, July 1, 1971 through June 30, 1972). Differences in normal reversion rates caused by mutagenic agents as judged by changes in thymidine kinase activity can be measured in order to evaluate and compare various mutagens. However, activity changes alone are of little help in understanding the mechanism of mutagenesis. Mutations in both control genes and structural genes could effect enzyme activity, but the latter influences the quantity of enzyme whereas the former can effect activity by alterations in kinetic and physical parameters. Although it is important to have a test system for the evaluation of mutagenic agents, it is equally as important to investigate mutagenesis at the molecular level. Hopefully, questions such as the specificity of different classes of mutagens will be better understood, and better and more reliable test procedures can be developed.

PROPOSED COURSE: To continue to develop purification techniques for enzymes in order to make comparative studies among the various mutant thymidine kinases.

Serial No.: NIEHS-PP-038
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Comparative Studies of Fetal and Adult δ-Aminolevulinic Acid (ALA) Dehydratase From Hepatic and Red Blood Cells"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Joseph B. Weissberg, M.D.

OTHER INVESTIGATOR: Peter E. Voytek, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	1.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To characterize changes in red cell ALA dehydratase during development of the guinea pig from the fetal to the adult stage and (2) to compare physical and kinetic parameters of red cell and hepatic ALA dehydratase.

METHODS EMPLOYED: Red cell ALA dehydratase activity has been measured in guinea pigs at different ages of prenatal and postnatal development. Partial purification of adult and fetal ALA dehydratase derived from both red cells and liver has been accomplished by ion exchange chromatography. Acrylamide gel electrophoresis, isoelectric focusing and the evaluation of kinetic parameters are among the techniques employed to investigate enzymatic changes as a function of age and tissue source.

MAJOR FINDINGS: Levels of red cell ALA dehydratase have been noted to be approximately three fold higher in the fetal than in the adult guinea pig. The fetal level of enzyme activity declines to that of the adult shortly after birth. Electrophoresis of the adult and fetal red cell and hepatic enzymes has shown identical migrations in acrylamide gels. Fetal and adult enzymes from both red cells and liver are also similar as demonstrated by K_M for the substrate. Increases in ionic strength have been shown to enhance equally the enzymic activity of red cell and hepatic ALA dehydratase. Differences in heat stability properties and in the kinetics of inhibition by hemin have been demonstrated for ALA dehydratase from different sources. Both adult and fetal red cell enzymes are unstable at 56°C, but a difference in their rates of inactivation has been

observed. The heat stability properties of ALA dehydratase from both adult and fetal red cells are in marked contrast to the adult hepatic enzyme which undergoes little or no loss in activity as a result of heat treatment. Adult red cell and hepatic ALA dehydratase have been shown to be similarly inhibited by hemin. However, the fetal red cell enzyme is more readily inhibited by this metabolite while the fetal hepatic enzyme is not appreciably inhibited.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The understanding of protein regulatory mechanisms in higher organisms will be aided by the elaboration of the biochemical differences between adult and fetal tissues and between differentiated tissues. The relevance of this approach is underscored by the identification in tumors of enzymes characteristic of fetal tissues. Hopefully, understanding of both normal and abnormal cellular development will be enhanced by the elucidation of enzymatic differences between fetal and adult cells. Lead is a known inhibitor of ALA dehydratase and more practical applications of the present studies extend to the area of environmental health. The differential effects upon adult and fetal enzyme systems of pollutants such as lead and numerous drugs in common usage need further study.

PROPOSED COURSE: Comparative studies of the physical and biochemical properties that characterize fetal and adult ALA dehydratase from hepatic and red blood cells will continue and will include investigations of the kinetics of inhibition by lead. Pilot studies of the red cell enzyme, purine nucleoside phosphorylase, have been undertaken and studies will be continued along the lines of those carried out with ALA dehydratase.

PUBLICATIONS

Weissberg, J.B. and Voytek, P.E.: Comparative studies of fetal and adult δ-aminolevulinic acid (ALA) dehydratase from guinea pig hepatic and red blood cells. Proceedings of the Federation of American Society of Experimental Biologists Meeting, Atlantic City, April, 1973.

Serial No.: NIEHS-PP-039
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Factors Affecting the Regulation of Hepatic Hematopoiesis During Mammalian Development"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: James S. Woods, Ph.D.

OTHER INVESTIGATOR: Vadiraja V. Murthy, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.0
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: (1) To elucidate the mechanisms governing the regulation of hepatic hematopoiesis during mammalian development through analysis of hepatic δ-aminolevulinic acid synthetase (ALAS) activity, (2) to isolate and biochemically characterize ALAS from fetal rat liver and (3) to study the effects of potentially porphyrogenic environmental agents on the regulation of hepatic ALAS during mammalian development.

METHODS EMPLOYED: ALAS activity was determined by two procedures. In liver homogenates ALAS activity was asssayed using the ion exchange chromatography technique described by D. Mauzerall and S. Granick (J. Biol. Chem. 219: 435, 1956) with liver homogenates prepared for incubation as described by H. S. Marver, D. P. Tschudy, M. G. Perlroth and A. Collins (J. Biol. Chem. 241: 2803, 1966). ALAS activity in subcellular fractions and at various stages of enzyme purification was measured using the procedure described by P. L. Scholnick, L. E. Hammaker and H. S. Marver (J. Biol. Chem. 247: 4126, 1972). In the latter case the incubation medium was modified to include 10^{-4}M GTP in addition to the prescribed substrates.

MAJOR FINDINGS:

(1) During the perinatal period of mammalian development, the activity of hepatic ALAS is significantly elevated with regard to that observed in normal adults, and the perinatal hepatic enzyme is refractory to the effects of agents

which induce or repress the synthesis of the enzyme in adult livers. Non-erythropoietic heme, the end product of the ALAS biosynthetic pathway, plays a complex role in the regulation of ALAS in adult rat liver by repressing enzyme synthesis, inhibiting its activity and preventing its incorporation by mitochondria. The regulation of hepatic ALAS during the perinatal period, however, is poorly understood. Analysis of the subcellular distribution of ALAS in fetal rat liver at 18 days of gestation reveals a striking 20:1 distribution of activity between mitochondrial and post-mitochondrial fractions. A 2:1 ratio is seen in adults. A 10-fold difference exists between fetal and adult mitochondrial enzyme activities. ALAS activity in all fractions of adult and fetal liver decreases rapidly after administration of cycloheximide, a specific inhibitor of ribosomal protein synthesis. The half-life of fetal mitochondrial ALAS is 47 minutes compared to 60-70 minutes observed in adults. D-chloramphenicol, a specific inhibitor of mitochondrial protein synthesis, also causes a rapid decrease in fetal mitochondrial ALAS activity but increases the activity of the post-mitochondrial fraction. These findings are consistent with the view that ALAS is synthesized in the cytoplasmic ribosomes and is subsequently incorporated into the mitochondria. The elevated enzyme activity observed in fetal mitochondria suggests that ALAS is incorporated into these organelles more rapidly or to a greater extent during the developmental stage. A facilitory role of heme in this process is suggested by the observation that administration of heme to fetal rats sustains and enhances the activity of ALAS in the mitochondrial fraction. In addition, heme delays the decrease in fetal mitochondrial ALAS activity after chloramphenicol administration and prolongs the half-life of the enzyme after treatment with cycloheximide. These observations are in direct contrast to those seen in adult rat liver. Whether heme plays a direct role in the mitochondrial incorporation of ALAS in the immature cell or acts indirectly through an effect on mitochondrial biogenesis is currently under investigation.

(2) ALAS from fetal rat liver has been purified approximately 20-fold. Procedures described for the isolation of the enzyme from soluble and mitochondrial fractions of porphyric adult rat liver were not directly adaptable to purification of the fetal enzyme, indicating that significant differences may exist in the properties of these proteins. The steps leading to increasing specific activity of the fetal enzyme include mitochondrial isolation, sonication of the mitochondrial suspension in Triton X-100, ammonium sulfate fractionation and gel filtration. The enzyme appears to exist as an aggregate and is relatively unstable in solution at the current stage of purification. Continuing work is planned with regard to further purification and subsequent comparison of the properties of this enzyme with those of ALAS already isolated from adult rat liver.

(3) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent inducer of hepatic ALAS in chick embryos. Studies designed to evaluate the potential porphyrogenicity of TCDD in mammals, however, have indicated that this compound does not induce ALAS in livers of several species. In addition, TCDD has no *in vitro* effects on ALAS isolated from rat liver, does not cause hepatic porphyrin accumulation and does not alter the subcellular distribution of ALAS in mammalian liver. Further experimentation is required to elucidate the nature of the differences determining species susceptibility to the porphyrogenic effects of TCDD and other compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The formation of non-erythropoietic heme is limited in mammalian liver cells by the activity of the enzyme, ALAS. The availability of heme, as a key component of mitochondrial and microsomal cytochromes, directly influences the capacity of the organism to maintain normal metabolic processes including the metabolism of drugs and other foreign agents. The regulation of ALAS during mammalian development is poorly understood. A clearer understanding of these processes, emerging from these studies, is required in order to predict and assess the effects on fetal and newborn animals of agents which alter the normal regulation of hematopoiesis in the mature organism.

PROPOSED COURSE: Studies related to the regulation of hepatic hematopoiesis during mammalian development will continue. The role of heme as a regulator of hepatic ALAS activity and of mitochondrial protein synthesis will be investigated in detail in adult and fetal animals. Further purification of ALAS from fetal rat liver will be pursued with the aim of biochemically characterizing the enzyme and comparing its properties with ALAS isolated from adult rat liver. Studies will continue with regard to elucidating the effects of potentially porphyrogenic environmental agents on the regulation of hepatic hematopoiesis.

Serial No.: NIEHS-PP-040
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Significance of Cleft Palate Induced by Chemicals in the Rat and Mouse"

PREVIOUS SERIAL NUMBER: NIEHS-CB-023,024 (July 1, 1970-June 30, 1971)

PRINCIPAL INVESTIGATOR: Herbert S. Posner, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNIT: Animal Science and Technology Branch

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To compare events that occur in the fetal rat and mouse during gestation in association with cleft palate formation after the maternal administration of five chemicals of four structural classes and (2) to submit one of these to a critical analysis.

METHODS EMPLOYED: The five compounds administered included both environmental compounds and drugs. These were chlorcyclizine, β-aminopropionitrile, cortisone, triamcinolone and N-nitroso-N-methylurea. Cleft palate was produced at incidences of 0.100% in a litter. The form of the cleft palate, the fetal length (as a measure of growth) and the cross-sectional area of the fetal thorax (as a measure of edematous enlargement of subcutaneous tissue which occurs in apposition to the oral region) were determined for the whole range of cleft palate incidence. The teratology of chlorcyclizine in the rat was submitted to detailed analysis in part by: (1) administering chlorcyclizine at an alternate time during gestation, (2) co-administration of two structurally different compounds that reduce the incidence of cleft palate, and (3) the comparison of fetuses with and without cleft palate occurring in the same litter.

MAJOR FINDINGS: Several patterns of fetal response occur. Chlorcyclizine in the rat resulted in the following pattern: fluid enlargement of the subcutaneous tissue over the fetal thorax which became greater as the incidence of cleft palate became greater, achieving 25-35% enlargement in association with 76-100% incidence of cleft palate; a larger proportion of wide (vertical-type)

cleft palate as the incidence of total cleft palate increased; and little reduction of fetal length. Only β-aminopropionitrile in the mouse and triamcinolone in the rat also showed enlargement of the fetal thorax, though this was at a maximum of 10% enlargement. In some of the cases, length was unaffected or minimally affected; in other cases, it was reduced as much as 25%. In some cases, both horizontal- and vertical-type cleft palates occurred; in others, either the horizontal- or vertical-type only occurred.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cleft palate is a rather frequent teratologic finding in experimental animals with drugs and environmental compounds. It is also found "spontaneously" in the human and it occurs in association with known genetic factors in the human and in experimental animals. It is clear from the present work that the significance of cleft palate that is obtained in teratogenesis tests cannot be decided without consideration of other parameters such as growth of the fetus, degree of subcutaneous fluid accumulated, and the form of the cleft palate at both low and high incidences of total cleft palate in the litters. Some of the information obtained could be applicable to cases of malformation in the human. Finally, several of the techniques developed should aid in further studies of the relationship between altered biochemical and physiological parameters and ultimate malformation of several types.

PROPOSED COURSE: The cleft palate aspect of the project was completed by the appearance of the fourth publication of the series. Other aspects of the project, for example the formation and the concomitants of fluid imbalances of several additional types will be investigated.

PUBLICATION

Posner, H.S.: Significance of cleft palate induced by chenicals in the rat and mouse. Fd. Cosmet. Toxicol. 10: 839-855, 1972.

Serial No.: NIEHS-PP-041
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Survival and Development of Mammalian Gametes and Embryos After In Vitro Exposure to Selected Chemicals and Conditions"

PREVIOUS SERIAL NUMBER: NIEHS-PT-025

PRINCIPAL INVESTIGATORS: R. E. Staples, Ph.D.; D. S. Elliott, Ph.D.; R. R. Maurer, Ph.D.; and L. A. Moustafa, Ph.D.

OTHER INVESTIGATORS: F. L. Schwartz, M.S.; C. D. Sloan, M.S.; J. P. Putnam, B.A.; R. G. Kellam, B.S.; E. L. Floyd, R.T.; and S. B. McCaffity

COOPERATING UNITS: None

MAN YEARS

Total:	3.4
Professional:	1.9
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVE: To determine the effects of various chemical and physical entities, acting alone and in combination, on subsequent development of mammalian gametes and embryos after exposure in vitro.

METHODS EMPLOYED: Gametes and embryos are recovered from donor animals and cultured in vitro for varying periods. Chemicals or conditions being tested are generally applied during the culture period. On occassion the donor may have been exposed and the treated gametes used for fertilization in vivo or for undergoing in vitro fertilization followed by additional culturing. Some preimplantation embryos are transferred to recipient females while others are examined for morphologic or chemical changes. Transferred embryos are usually examined near term for external visceral and skeletal malformations, but when indicated they may be studied at earlier stages of gestation or even following birth.

MAJOR FINDINGS: A reliable method for culturing early cleavage stage embryos of the mouse, rabbit and rat to the blastocyst stage has been instituted. In addition, both cultured and non-cultured embryos are being transferred to recipient females on a routing basis. About one-half of the transferred blastocysts remain viable to term and appear to have developed normally. The species

studied differ in the amount of atmospheric pressure required for optional development to the blastocyst stage in vitro. In so far as the investigators are aware, cultured rat embryos have not previously been transferred to recipient females and carried to term.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Techniques permitting development of preimplantation embryos in vitro with retention of viability to term after transfer to the uteri of recipient females makes possible direct exposure of mammalian embryos to test agents, thereby permitting the investigator to distinguish between direct and indirect effects of environmental insults on embryo development. Information will also be gained that will increase our understanding of embryonic requirements for development.

PROPOSED COURSE: We will continue to improve culture and transfer methodology for those species that we now routinely culture and will attempt to perfect techniques that will permit the successful culture of the embryos of additional species, e.g. hamster. Specific substances and conditions are being selected for study by the system described, e.g. thalidomide, captan, DMSO, and microwave irradiation.

Serial No.: NIEHS-PP-042
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Low Temperature Storage of Mammalian Embryos"

PREVIOUS SERIAL NUMBER: NIEHS-PT-026

PRINCIPAL INVESTIGATOR: R. R. Maurer, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D.; F. L. Schwartz, M.S.; and H. L. Bank, Ph.D., Department of Anatomy, Duke University

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: (1) To establish methodology for the storage of viable mammalian embryos, (2) to verify existing literature reports of freezing mouse embryos, (3) to assess the effects of freezing and low temperature storage on subsequent *in vitro* and *in vivo* development, (4) to determine if mammalian embryos of various species respond similarly to freezing and low temperature storage, and (5) to provide a bank of stored embryos.

METHODS EMPLOYED: Embryos are recovered by conventional methods from mice on Day 3 of gestation and from rabbits on Day 2 of gestation. The embryos are washed with and placed in a phosphate buffered saline solution fortified with pyruvate, bovine serum albumin and DMSO. Embryos are frozen slowly (0.5 - 1.5°C/min), and the temperature gradually reduced to -80 to -110°C before the embryos are placed in liquid nitrogen (-196°C). After various periods of storage, the embryos are thawed slowly (4 to 10°C/min), washed and placed in culture for 48 hours. After culturing, the resulting mouse blastocysts are transferred to the uterus of Day 3 recipient mice. Some of the recipients are allowed to parturate. The fetuses from the remaining recipients are removed by cesarean section one or two days before normal delivery and examined for teratological changes.

MAJOR FINDINGS: The original report on the freezing of mouse blastocysts was not repeatable in our laboratory or by the original investigator. Subsequent alterations in experimental procedures reported by the same author regarding

the freezing of 8-celled mouse embryos have been successfully duplicated. The incidence of survival of frozen mouse embryos after thawing, culturing for 48 hours and subsequent transfer to recipient females is comparable to that obtained after the transfer of cultured control embryos. Viable young have been obtained after being stored as 8-celled embryos at -196°C for 30 days. F_1 progeny have been produced from frozen embryos. Eight-celled rabbit embryos have also been frozen and thawed successfully as judged by subsequent development *in vitro* to the blastocyst stage.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The freezing and storage of embryos can greatly facilitate experimental studies with regard to ontogenesis and teratology by providing a readily available source of material. The fact that embryos can be frozen and subsequently thawed after a prolonged storage period might be a valuable way to preserve rare (mutant) mammalian strains. The general techniques being developed can also provide further information regarding the freezing of human tissues and organs.

PROPOSED COURSE: The study of effects of freezing embryos on subsequent development will be continued. Long-term storage and subsequent ontogenesis will be further investigated in mice and other mammals.

Serial No.: NIEHS-PP-043
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effect of Direct Application of Test Agents to the Mammalian Embryo on Subsequent Viability and Development"

PREVIOUS SERIAL NUMBER: NIEHS-PT-027

PRINCIPAL INVESTIGATOR: Laila A. Moustafa, Ph.D.

OTHER INVESTIGATORS: Robert E. Staples, Ph.D., and Carol D. Sloan, M.S.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	0.5
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVE: To investigate the susceptibility of the developing mammalian embryo to test chemicals applied directly.

METHODS EMPLOYED: A micrurgical technique recently developed by the principal investigator has been adapted for direct administration of test agents into mammalian blastocysts. After micromanipulation, blastocysts are either prepared for study by histochemical and cytological techniques or transferred into recipient females. Pregnant females are sacrificed prior to natural term for embryopathic observations. To follow the uptake of tested agents radio-labeled compounds are used.

MAJOR FINDINGS: A micrurgy system has been established for several mammalian embryos. Embryos are now being successfully micromanipulated, cultured and transferred into foster mothers with no apparent adverse effects on development. Thalidomide is currently under study to determine whether malformation will result from local application.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The technique described permits study of the direct effects of various compounds on the developing mammalian embryo. This technique provides a means of by-passing maternal influences such as metabolism which affects the distribution of test agents. It is hoped that interspecies variability in embryonic response will be minimized by these procedures, thereby, increasing the predictability

in regard to what might be expected upon exposure of human embryos to a given concentration of the parent test agent.

PROPOSED COURSE: Investigations of classic teratogens and environmental agents will be continued to determine the potential of this approach in resolving some of the problems involved with the extrapolation of experimental results to humans.

Serial No.: NIEHS-PP-044
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effect of Environmental Components on Mammalian Ontogenesis After Parental Exposure"

PREVIOUS SERIAL NUMBER: NIEHS-PT-028

PRINCIPAL INVESTIGATORS: R. E. Staples, Ph.D.; D. S. Elliott, Ph.D.; R. R. Maurer, Ph.D.; and L. A. Moustafa, Ph.D.

OTHER INVESTIGATORS: F. L. Schwartz, M.S.; C. D. Sloan, M.S.; R. G. Kellam, B.S.; J. P. Putnam, B.A.; E. L. Floyd, R.T.; G. F. Stranzinger, Ph.D.; and S. N. Baksi, Ph.D.

COOPERATUNG UNITS: None

MAN YEARS

Total:	5.7
Professional:	3.3
Other,	2.4

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the teratogenic potential of single or combined environmental components in mammals by studying pre- and postnatal survival and development of the offspring of exposed individuals, (2) to determine the effect of such components on other reproductive processes, and (3) to compare *in vivo* teratogenicity to that observed after exposure of blastocysts in culture.

METHODS EMPLOYED: Test agents are given to pregnant females of at least two species (e.g. mouse and rabbit) by two or more routes (e.g. per os, subcutaneous, intrauterine application) for various periods of ontogenesis. Doses are selected on the basis of preliminary study and which produce a given pharmacological response (LD_5 for the female, maximum tolerated dose, comparable blood levels, 50% fetal mortality, etc.). Stresses or disease states that may be elicited before mating are also studied for teratogenic potential either alone or in combination with other environmental components. Results are compared to those obtained after *in vitro* application of the same test agents. An attempt is made to compare the sensitivity of the test systems. Qualitative or quantitative differences or similarities in teratogenic response are noted. Resulting offspring are examined for alterations in the incidence of external, visceral and skeletal changes shortly before term. When indicated, both males

and females are exposed to the test agent before breeding and the offspring may be studied at other stages of gestation for morphologic changes. Postnatal studies determine differences from controls with respect to survival, growth rate, behavioral changes and functional capability. Karyotyping and chromosomal binding techniques are being applied to embryonic, fetal and adult cells and tissues of the rabbit in an effort to detect aneuploidy and reciprocal translocations.

After exposure of males and females to test agent(s), various reproductive parameters are examined for impaired function if indicated by the results of preliminary studies.

MAJOR FINDINGS: DMSO is known to readily penetrate biological membranes; however, species appear to differ in teratogenic response to DMSO administration. To date, we have demonstrated teratogenicity in the mouse (i.p. and s.c.) and hamster (i.p.) but have not in the rat (s.c.), even though dosage was sufficient to induce extensive embryo lethality. The rabbit (s.c.) is also resistant to the teratogenic effect of DMSO.

Thyroparathyroidectomy on Day 1 of pregnancy in the rat resulted in increased embryo lethality by Day 20 of gestation, but a teratogenic response was not evident.

Karyotyping and chromosomal banding patterns have been prepared for evaluation of embryos at the 8-cell and blastocyst stage, various tissues of the 16-day 28-day fetus, and the bone marrow and leukocytes in the adult rabbit.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Through the effort described, the teratogenic potential of environmental components will be evaluated. The teratogenic response of various species to agents being tested *in vivo* will be compared to the response after exposure of preimplantation embryos during culture. Our hope is that a means of testing can be derived that will improve extrapolation of experimental results to man.

PROPOSED COURSE: Specie differences in response to DMSO are being studied further. Other chemicals being tested are thalidomide and captan. The teratogenic potential of thyroidectomy in rats performed at the ages of 24 and 40 days as well as on the day of breeding is being investigated. The banding patterns of chromosomes after Giemsa staining are being evaluated to determine whether the patterns change during development among tissues within individual rabbits and among rabbits of different breeds.

Serial No.: NIEHS-PP-045
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Survival and Development of Mammalian Embryos After Cleavage Inhibition In Vitro"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: D. S. Elliott, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D., and E. L. Floyd, R.T.

COOPERATING UNITS: None

MAN YEARS

Total:	0.9
Professional:	0.5
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: (1) To verify existing literature reports of reversible cleavage inhibition of rabbit embryos by addition of progesterone during embryo cultures, (2) to assess the effects of reversible cleavage inhibitors on subsequent survival and development of the embryo, (3) to test procedures for inhibiting cleavage such as cold storage, and (4) to compare the effects of cleavage inhibition among various mammalian species.

METHODS EMPLOYED: Embryos are recovered from females at the desired cell stage and are subjected to the cleavage inhibitor in vitro for varying periods of time. The inhibitor is then removed, and the embryo is allowed to develop for a short period of time under the usual culture conditions. Those embryos that undergo development in culture are transferred to pseudopregnant recipients. Fetuses are removed by cesarean section one or two days prior to expected parturition and examined for teratological changes.

MAJOR FINDINGS: This project has only recently been initiated, and there are no significant findings to report at this time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One effect that various environmental factors have on the mammalian embryo is to inhibit cleavage. It is therfore of interest to study the mechanisms of cleavage inhibition and its effect upon subsequent embryo survival and development.

PROPOSED COURSE: Since these studies have only recently been undertaken they will be pursued as outlined.

Serial No.: NIEHS-PP-046
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Uterine Luminal Secretions and Embryonic Development"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. R. Maurer, Ph.D.

OTHER INVESTIGATORS: H. M. Beier, Ph.D., Anatomical Institute, University of Kiel, W. Germany; and F. S. Schwartz, M.S.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the biologic significance of uterine luminal secretions present during early pregnancy, (2) to determine if the embryo can produce specific proteins, e.g. uteroglobin, and (3) to determine whether quantitative or qualitative changes in luminal secretions occur when animals are placed under stress or subjected to environmental factors.

METHODS EMPLOYED: The effects of uterine luminal proteins on cultured embryos are being studied. The effects of these proteins which are isolated from 5-6 day pseudopregnant rabbits are compared to these associated with the proteins which are normally part of the incubation medium. To determine whether rabbit embryos *per se* can produce uteroglobin, 2- and 4-celled embryos are cultured for 96 hours in a medium containing bovine serum albumin and then the embryos and the medium are analyzed electrophoretically for uteroglobin. In addition, rabbit uterine luminal fluids are being collected on Days 4 to 6 of gestation and the proteins are examined electrophoretically to determine the normal composition and to determine the effect of various environmental factors on these constituents.

MAJOR FINDINGS: Eight-celled rabbit embryos cultured for 96 hours in a medium containing fractionated uterine proteins as the macromolecule did not develop blastocoeles. To date, uteroglobin has not been detected in culture medium or in blastocysts after 2- and 4-celled embryos have been cultured for 96 hours.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elucidation of the biologic significance of uterine secretions would add to our knowledge of the processes necessary for the further development of preimplantation embryos. If environmental components can be shown to alter the composition of uterine secretions, additional experimental efforts might demonstrate such alterations to be at least partially responsible for the high level of embryonic mortality seen in virtually every mammalian species at the time of implantation. These findings might also be of importance in the development of newer methods for population control.

PROPOSED COURSE: Uterine luminal constituents will be tested further to seek a component that will initiate blastocoele formation. The uterine luminal proteins obtained from control and stressed females will be examined for qualitative and quantitative differences.

Serial No.: NIEHS-PP-047
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "An Investigation of the Molecular Bases for Modulations Plasma Membranal ($Na^+ + K^+$) ATPase Behavior in Response to Chlorinated Hydrocarbon Inhibitors, Detergents, Phospholipids, Drugs and Hormones"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-008

PRINCIPAL INVESTIGATOR: William E. Wilson, Ph.D.

OTHER INVESTIGATORS: Charles W. Sharp, Ph.D.
Nancy K. Wilson, Ph.D., Animal Science and Technology Branch

COOPERATING UNITS: None

MAN YEARS

Total:	2.7
Professional:	1.7
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVE: To attempt to ascertain the mode of action of chlorinated hydrocarbons (DDT and PCBs) and of various detergents and inhibitors of the ($Na^+ + K^+$) ATPase systems from several tissue sources. Inhibition of the ($Na^+ + K^+$) ATPase systems in intestine and gills has been claimed by Kinter and coworkers to be responsible for the toxicity of chlorinated hydrocarbons to eels. An understanding of the mode of toxicity of the chlorinated hydrocarbons to the ($Na^+ + K^+$) ATPase systems could permit us to obtain (a) a realistic model for the toxicologically significant biological alterations which result from exposure of a mammalian cell to these types of compounds and (b) a more comprehensive understanding of the phospholipid dependence of ($Na^+ + K^+$) ATPase systems in various mammalian plasma membranes.

METHODS EMPLOYED: Various spectrophotometric, chromatographic, centrifugation, enzyme analytical and electron microscopy techniques are used.

MAJOR FINDINGS: We have been primarily concerned with comparing responsiveness on three ($Na^+ + K^+$) ATPases to a variety of chlorinated hydrocarbons, detergents, phospholipids, phospholipase A and fatty acids. The responsiveness of beef brain and of rabbit kidney ($Na^+ + K^+$) ATPases to DDT, Aroclor 1221, Aroclor

1254, several anionic detergents, several fatty acids and phospholipase A is similar from the point of view of the temporal and/or concentration dependencies of enzyme inactivation as well as from the point of view of the selectivity among phospholipids in protecting against and/or reversing such inactivation. The eel electroplax ($Na^+ + K^+$) ATPase differs from the two mammalian enzymes in at least two ways, namely: (a) the eel enzyme appears to be nonselectively stabilized by phospholipids and (b) the concentration dependencies for both inactivation and phospholipid restabilization of the eel enzyme differ significantly from those for the mammalian enzymes. However, behavior of the eel enzyme was consistent with the behavior of the mammalian enzymes in one important regard, namely, the phospholipid selectivity for protection against chlorinated hydrocarbons, deoxycholate and phospholipase A were identical.

The results from studies of the brain and kidney ($Na^+ + K^+$) ATPases provide positive support for our contention that chlorinated hydrocarbons such as DDT, Aroclor 1254 and, possibly, Aroclor 1221 effect their inactivation by interfering with phospholipid stabilization of these enzymes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The sodium pump ($Na^+ + K^+$) ATPases represent hormone-responsive (e.g., glucagon, angiotensin, insulin and epinephrine) plasma membranal systems which perform several primary functions (e.g., alkali metal ion translocation and generation of membranal electrical potential gradients) and which seem to be involved in several secondary cellular functions (e.g., regulation of acetylcholine release in parasympathetic nerves and, possibly, translocation of amino acids or sugars). Several other plasma membranal systems also have been shown to depend upon phospholipids for normal stabilization; however, the nature of a membranal enzyme's phospholipid dependency has received most attention in the case of the ($Na^+ + K^+$) ATPases. Our focus on obtaining a more comprehensive understanding of those aspects of ($Na^+ + K^+$) ATPase behavior which are influenced by and/or are dependent upon phospholipids will permit us to obtain a clearer understanding of the extent(s) to which perturbations on plasma membranal phospholipid may be tolerated by this particular enzyme system. It is not unreasonable that this type of information will be of rather wide interest in that it may permit anticipation of effects of lipophilic environmental agents on this and other plasma membrane-associated functions (e.g., adenyl cyclase behavior).

PROPOSED COURSE: Based on our previous studies, several questions which should be answered include: (1) Is interference with phospholipid stabilization of ($Na^+ + K^+$) ATPases on adequate rationalization of the mode of toxicity of chlorinated hydrocarbons for membranal enzyme systems from other tissues? (2) Does ($Na^+ + K^+$) ATPase inactivation result from enzyme interaction with the monomeric form, the micellar form or both forms of DDT or the PCBs? (3) Could the phospholipid selectivity relative to chlorinated hydrocarbon inactivation of the enzyme be obtained with a "pure" ($Na^+ + K^+$) ATPase preparation? (4) How general is the requirement that membranal ($Na^+ + K^+$) ATPase stabilization be effected by acidic phospholipids?

Other questions which could be of interest include: (1) Could the modulation of the behavior of mammalian (or other) ($Na^+ + K^+$) ATPases by hormones be influenced to a significant extent by DDT, PCBs, fatty acids or various drugs? (2) Could inactivation of placental and/or fetal ($Na^+ + K^+$) ATPases be related

(causally or otherwise) to fetal edema in response to various drugs and/or bacterial toxins?

We plan to seek answers to at least several of the above-mentioned questions by extending our investigations of ($Na^+ + K^+$) ATPase behavior to tissues (other) than brain and kidney) such as intestine, cardiac muscle, lung, liver, placenta and, perhaps, various fetal tissues. The aspects of ($Na^+ + K^+$) ATPase behavior to be investigated include: (1) phospholipid selectivity for enzyme stabilization and (2) determination of enzyme responsiveness to DDT, DDE, PCBs, fatty acids, detergents, several hormones, cyclic AMP and/or several protein bacterial toxins (if obtainable in satisfactory form).

PUBLICATION

Wilson, N.K. and Wilson, W.E.: Complex equilibria involving DDT and model compounds for phospholipid and protein. Sc. Total Environ. 1: 245-251, 1972.

Serial No.: NIEHS-PP-048
1. Pathologic Physiology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Purification of the Beef Brain ($Na^+ + K^+$) ATPase System"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-040

PRINCIPAL INVESTIGATOR: Charles W. Sharp, Ph.D.

OTHER INVESTIGATOR: William E. Wilson, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVE: The primary objective is to obtain a pure preparation of a membranal ($Na^+ + K^+$) ATPase to ascertain whether this enzyme requires specific phospholipids for normal stabilization. Such knowledge is imperative for an understanding of phospholipid selectivity in reversing chlorinated hydrocarbon (CH) inhibition of this enzyme and to examine the relative interaction of other plasmic membranal components with this system.

METHODS EMPLOYED: Sepctrophotometry, ultracentrifugation, polyacrylamide electrophoresis, radioactive tracer techniques, cryogenic techniques, molecular distillation and various chromatographic techniques are used.

MAJOR FINDINGS: Phosphatidyl serine (PS) selectively protects against or reverses the effects of DDT and mixture of polychlorinated biphenyls contained in arochlor 1254 on the $Na^+ + K^+$ ATPase activity of at least three different tissues (beef brain, rabbit kidney and fish brain). A similar selectivity exists for the protection or reversal of a more purified form (microsomal) of this enzyme from inactivation by phospholipase A or deoxycholate. The widespread occurrence of the selective protective effect of PS lends more credence to the findings and allows a wider selection of tissue as a source for the purification of the enzyme. Preliminary efforts have been made to obtain a suitable chromatographic system for the purification of this enzyme system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At present, there are very few membranal enzyme systems which have been purified.

It is also difficult to rationalize biological effects of environmental agents at the molecular level because appropriate responsive membranal components are not available for study. Thus, an effort is required to attempt to obtain such purified components.

PROPOSED COURSE: Efforts to attain a satisfactory support utilizing affinity chromatography in the purification of this enzyme will continue. This includes the synthesis of several different types of ATP modified supports and possibly other appropriate analogs of phosphatidyl serine or ouabain. Although the approach of affinity chromatography will be emphasized, the enzyme will be treated with detergents, chaotropic agents and other agents before fractionation. Ammonium sulfate precipitation, rate-zonal centrifugation and/or anion-exchange chromatography will also be used according to documented methods of purification for the tissue used.

The purified enzyme will be extracted by different procedures, preferably at low temperatures, and anlayzed prior to or following readdition of the phospholipids in order to evaluate the specificity of the phospholipid requirements. Other techniques of examination of the interaction of DDT specifically with the $Na^+ + K^+$ ATPase (such as molecular probes) are also being considered.

PHARMACOLOGY AND TOXICOLOGY BRANCH

PHARMACOLOGY AND TOXICOLOGY BRANCH
Summary Statement

The Pharmacology and Toxicology Branch carries on research in four major areas: (1) Toxication-Detoxication Systems, (2) Pharmacokinetics, (3) Lung Physiology, Biochemistry and Pharmacology, and (4) Inhalation Exposures and Toxicology. These research programs seek to identify potential toxic effects of pollutants or environmental components, or interactions of these with other materials to which we are exposed - e.g. - chemicals, food additives, drugs. Any effects seen are studied with regard to their specificity (seen in one or several animal species, strain, age or sex), and mechanism (at least far enough to perhaps predict, or alter serious toxicities). For example, an intersectional program between Toxication-Detoxication Systems, Pharmacokinetics, Lung Physiology and Inhalation Toxicology is studying the uptake, storage, metabolism and release of chemicals by the lung. This program has identified several pairs of chemicals and drugs which may interact at the processes of uptake and release of chemicals by the lung and is now studying dose-response, time-response, and mechanism of such interactions to better decide which are most likely to be practically important and dangerous.

Since last year major changes in organization have occurred. The program in reproduction and ontogenesis (including chemical teratogenesis) has been transferred to the Pathologic Physiology Branch and segments of the Chemistry and Pathologic Physiology Branches whose chief research interests lay in toxication-detoxication systems or pharmacokinetics have joined the Pharmacology and Toxicology Branch.

Program

Toxication-Detoxication Systems: Research in this area is comparing chemical metabolizing systems in organs in contact with the environment - e.g. - liver, lung, and gut. The liver has long been thought of as the major, if not only, body organ capable of metabolizing (and thereby toxifying or detoxifying) chemicals or drugs. Our efforts are directed at comparing hepatic with extrahepatic systems and their responses to environmental components and other common chemicals including drugs. We are especially interested in the balance of toxication and detoxication systems in various organs and in factors that may upset this balance. Some recent findings indicate that the lung may differ markedly from the liver in such balance and in response to factors that can change this balance. Development of xenobiotic metabolism in the newborn animal is of great interest when different tissues having such systems show different developmental patterns, as also seems to be true in liver vs. lung.

Pharmacokinetics: Two organs are the focus of efforts - the lung and the liver. The processes of uptake, storage, metabolism, and release or excretion

of chemicals by these organs have been subjected to rate studies especially with the lung. At least two different processes of chemical uptake by lung have been identified, and competition by chemicals for one of these processes has been used to identify possible toxic interactions based on this competition. Interestingly, a series of structurally-similar herbicides was studied and none of its members became a candidate for interaction with other chemicals or drugs at the uptake step in the lung. A toxic interaction of great interest would result from sudden release from the lung of a drug or chemical stored there in "inert" form. Such a reaction seems possible with certain pairs of chemicals now being studied intensively by this section.

Lung Physiology, Biochemistry and Pharmacology: Studies of lung functions in intact animals and comparisons with the isolated perfused lung attempt to identify early warning systems for chemical damage to the lung. Other studies concern the synthesis, storage and release of lipids in the lung including the processes involving lung surfactant and prostaglandins. The uptake and incorporation of chemicals into lung enzyme systems (as opposed to simple storage as studied by the pharmacokinetics group) is being studied as a model of relatively irreversible chemical effects on the lung.

Inhalation Toxicology: A variety of components of aerosol spray cans have been investigated for their toxic effects in rats, rabbits and hamsters. Work continues on the effects of aluminum chlorhydrin on the lung. A major program involves the toxic actions of inhaled chemicals as these are affected by treatment with other chemicals or drugs. The interactions of organic solvents (inhaled) in animals pretreated with barbiturates, tranquilizers, or pollutants has been a continuing study. Similarly the interactions of dichloromethane (a very common solvent) with CO and CCl_4 are being investigated. The effects of freons (aerosol spray propellants) on the cardiovascular system and on the toxicity of other inhaled materials is a new study this year.

General: Many interactions occur and are promoted between these sections of the Pharmacology and Toxicology Branch. A recent study on PCB distribution in the body involved scientists from the Toxication-Detoxication Section (effects of PCB's on lung and liver detoxication systems), Pharmacokinetics (rates of uptake and redistribution in various body tissues), and Lung Physiology and Biochemistry Section (site of storage of PCB's in lung seemed to involve lipids). Such "team" efforts allow an interdisciplinary and integrated study which is seldom found in the universities or elsewhere in my experience.

Facilities

Due to personnel restrictions we have not been able to make maximal use of the inhalation facility. The present staff under Dr. Drew's direction is able to manage the operation of only four chamber rooms. Sometime within the next year we hope to be able to award a contract for some of the chronic, long-term and low-level exposures to environmental contaminants that are so consuming of manpower. Another need is for consolidation of some laboratories now split between two and three buildings - especially the scientists in the Pharmacokinetics Section. Remodeling of three labs in Building 4 and Building 14 has helped make some space more efficient, but some of the new people added to the Branch (see below) are still so physically isolated as to make their

contributions to the overall program (in Pharmacokinetics) far less than optimal.

Personnel

Personnel added this year included transfers from other Branches as well as some temporary and special (non-permanent) program participants. The transfers include Drs. H. B. Matthews (Senior Staff Fellow), H. Mehendale (Staff Fellow), P. R. Chen (Visiting Fellow), and Ms. M. Fields (Biological Laboratory Technician) from Chemistry Branch plus Drs. G. W. Lucier (Senior Staff Fellow), S. W. Hwang (Staff Fellow), B. R. Sonawane (Visiting Fellow), and Ms. O. McDaniel (Biological Laboratory Technician), and P. Singletary (Stay-in-School) from the Pathologic Physiology Branch. Other new additions at the professional level included Dr. L. G. Hart (Supervisory Pharmacologist, replacing Dr. Gram), Dr. C. Harper (Staff Fellow), and Dr. G. J. Taylor (Staff Associate). New Visiting Fellows included Drs. M. O. James, F. Law, and R. D. Pickett. Full-time technicians were Mr. G. L. Foureman (replacing Clyde Williams), and Ms. D. Howell (replacing Sandra Gipson). Part-time technicians were Ms. L. Gilmore and Ms. J. Van Anda. Special program members included Mr. M. Carlone, Mr. Z. McCoy (Upward Mobility), and members of the Stay-in-School program including Mr. W. J. Lee, Mr. D. G. Prince, Ms. E. Simpson, Mr. F. Suddreth, Mr. W. Taylor, and Mr. M. Clemons. Branch losses in personnel included the entire section headed by Dr. Staples - this was transferred to Pathologic Physiology.

At present the Branch is overwhelmingly staffed with non-tenured and non-full-time people. The turnover is especially large among the special programs members. Such turnover is a tremendous drain on professional staff time because to be effective, even part-time people must be trained, and this training must be done by the full-time and professional laboratory staff. Though this non-tenured nature of the bulk of the Branch personnel may be the best arrangement possible now, it is a "terrible way to run a railroad." The Stay-in-School, Upward Mobility, etc., programs are all extremely worthwhile, but their demands on an already tightly-stretched staff are tremendous. The dependence on temporary laboratory staff is also excessive in terms of program planning - entire programs in the Branch are now dependent on temporary technical help, and therefore are subject to sudden loss when this help has to leave (e.g. - Dr. Philpot's entire research effort depends on Mr. Simmons who leaves this summer).

The ability to construct high quality and very productive research programs in the face of these kinds of odds is a tribute to quality of my staff - they are doing a tremendous job in my opinion.

Other Activities

Several of the professional staff serve on committees, etc., helping other organizations such as local universities, other Institutes of NIH, other Government agencies and professional societies. The listing below is meant to be complete, but undoubtedly suffers some omissions.

Dr. Fouts is an Adjunct Professor of Pharmacology at the School of Medicine, University of North Carolina at Chapel Hill and lectures in the graduate and

medical school courses in pharmacology. He is a member of the Dental Research Center's Scientific Review Committee (UNC, Chapel Hill). He is also an Adjunct Professor of toxicology in the School of Agriculture and Life Sciences, North Carolina State University at Raleigh, and serves on the Toxicology Advisory Committee of the Department of Entomology at that University. Editoral Board membership includes Chemico-Biological Interactions, Xenobiotica, and the NIEHS experimental journal, Environmental Health Perspectives. He is a regular reviewer for the Journal of Pharmacology and Experimental Therapeutics, Biochemical Pharmacology, Toxicology and Applied Pharmacology, and the Journal of Laboratory and Clinical Medicine. Committee memberships include the Committee on Environmental Pharmacology of the American Society for Pharmacology and Experimental Therapeutics; Committee on Anticonvulsant Drugs and the Epilepsy Advisory Committee of the National Institute of Neurological Diseases and Stroke, NIH; Scientific Advisory Board of the National Center for Toxicological Research, Arkansas; and organizer and Chairman of the Symposium on Environmental Pharmacology at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics (1973 at Michigan State University). Visiting lectures have been given at the Second International Symposium on Microsomes and Drug Oxidations (Stanford University, July 1972), Food and Drug Directorate (Ottawa, Canada, October 1972), University of Cincinnati (November 1972), Virginia Tech (March 1973), and University of Kentucky (April 1973).

Dr. Robert T. Drew is a member of the Environmental Health Committee of the Society of Plastics Industries and an Advisory Committee to the lung cancer program of the chemical carcinogenesis group at the National Cancer Institute, (NCI, NIH).

Dr. H. B. Matthews is program chairman of the Toxic Agents Seminar, North Carolina State University.

Dr. G. W. Lucier is Managing Editor of the NIEHS experimental journal, Environmental Health Perspectives.

Dr. R. P. DiAugustine is a member of the Editoral Board of the NIEHS.

Dr. L. G. Hart has served as a consultant to the ad hoc Biochemical Pharmacology Review Committee of the Experimental Therapeutics Branch of the National Cancer Institute (NCI, NIH).

Dr. J. R. Bend was an invited speaker at the Second International Symposium on Microsomes and Drug Oxidations at Stanford University, July 1972.

Dr. Harvey Cohen was an invited speaker at a conference on "Metalloenzymes" sponsored by the Molecular Enzymology Group and the British Biophysical Society in London, England, September 1972.

Dr. Curtis Harper is an adjunct instructor of pharmacology at the School of Medicine, University of North Carolina at Chapel Hill and assists in instruction for medical and graduate students in pharmacology.

Serial No.: NIEHS-PT-003
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Effects of Drugs and Chemicals on the Inhalation Toxicity of Chemical Solvents"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: R. T. Drew, Ph.D., and C. Harper, Ph.D.

OTHER INVESTIGATORS: B. Gupta, Ph.D., J. R. Lo, Ph.D., R. P. Roth, Ph.D., G. J. Taylor, M.D., J. Zinkl, Ph.D., Frank Harrington, Emile Lores, B.S., Mike Riley, and Joanna Schroeder

COOPERATING UNITS: Animal Science and Technology Branch (Histology), Biometry Branch

MAN YEARS:

Total	3.9
Professional:	2.1
Other:	1.8

PROJECT DESCRIPTION

OBJECTIVES: Liver is known to contain microsomal toxication and detoxication enzymes that are inducible by certain drugs and other chemicals. There are suggestions that analogous lung enzymes are also inducible. Detoxication of some organic solvents is catalyzed by some of these microsomal enzymes. This study has the following objectives:

1. Design a reliable method for measuring the enzymatic degradation of benzene by lung and liver microsomes.

2. Determine if pretreatment of animals with drugs and other chemicals which modify liver and/or lung microsomal enzymes will alter the acute and chronic inhalation toxicity of selected organic solvents.

3. Determine whether pretreatment (by inhalation) by common solvents affects their enzymatic degradation in lung and liver.

4. Study the species differences in benzene metabolism by rat, rabbit and hamster microsomal enzymes from both lung and liver. Measure the biochemical parameters that define the metabolism in both tissues of the three species.

5. Investigate some of the aspects of the biochemical conversion of dichloromethane to carbon monoxide (R. P. Roth, Ph.D.).

6. Determine if inhalation exposure to known hepatic microsomal enzyme inducers affects analogous lung enzyme activities.

METHODS EMPLOYED: Inhalation exposures are carried out in chambers specifically designed for such studies. They are operated under dynamic conditions of air flow and atmospheric concentrations of specific compounds are monitored at frequent intervals. Biochemical assays are carried out in routine fashion. Dichloromethane exposures to rabbits are carried out under dynamic conditions in a specially designed nose exposure unit.

MAJOR FINDINGS:

1. A reliable, reproducible method has been developed to assay for benzene metabolism by lung and liver microsomes.

2. Animal pretreatment by known hepatic microsomal enzyme inducers increases metabolism of benzene by liver microsomes from both the rat and the rabbit while not affecting metabolism of benzene by lung microsomes. These enzyme inducers do not affect the acute toxicity of inhaled or injected benzene in rats.

3. Three 4-hour inhalation exposure to benzene (4,000 ppm) induces benzene metabolism in rat liver but not in lung.

4. Chronic exposure of rats to benzene (1,700 ppm) (6 hrs/day, 5 days/week) causes minimal induction of benzene metabolism in liver microsomes with no change in lung microsomal metabolism of benzene. Phenobarbital does not protect against the leucopenic action of inhaled benzene under these conditions but does induce hepatic microsomal metabolism of benzene to phenol.

5. The inter-species comparison of benzene metabolism is summarized in the following table

	Specific activity (nmoles/min/mg protein)		
	Rabbit	Hamster	Rat
Lung	3.2	2.5	0.7
Liver	2.3	2.9	0.9
Lung/liver	1.4	0.87	0.78

6. Dichloromethane studies (R. P. Roth, Ph.D.) - Preliminary studies in rabbit have shown that (1) multiple exposures to dichloromethane do not increase the carboxyhemoglobin levels indicating that there is not a self-inducing effect of dichloromethane on its metabolism to CO, (2) pretreatment by known inducers of hepatic microsomal enzymes does not increase the carboxyhemoglobin level and yet (3) pretreatment by CCl_4 does decrease the carboxyhemoglobin levels obtained as a result of exposure to dichloromethane. CCl_4 can poison both liver and lung microsomal systems metabolizing chemicals.

7. Inhalation exposure to phenobarbital aerosols induces hepatic microsomal metabolism of benzene but does not alter pulmonary microsomal metabolism of this solvent.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In today's society the number of people taking drugs either prescribed by a physician or illicitly is substantial. Some of these compounds stimulate liver microsomal enzymes, and possibly lung system, thus increasing the rate at which certain chemicals and/or other endogenous substrates are metabolized. These studies are designed to better define the toxic responses to selected solvents and to determine if the toxicity of these solvents can be altered by pre-exposure to themselves or to other drugs. The effect of the route of administration is also being studied. Benzene has been chosen because of its widespread industrial and household use and because it is now being proposed for use instead of Tetraethyl lead in increasing gasoline octane ratings.

The recent findings that humans who are exposed to dichloromethane have elevated carboxyhemoglobin has generated considerable interest. Studies designed to elucidate the nature and site of this reaction have been started. Should this metabolism occur exclusively in lung, a model of pulmonary metabolism analogous to the hexabarbital sleeping time model for hepatic metabolism could be used to study effects of a variety of agents.

PROPOSED COURSE: These relationships, as well as others will continue to be investigated. Additional effort will be directed toward elucidation of paraxylene metabolism. Studies with other benzene derivatives such as nitrobenzene and chlorobenzene will be initiated. A paper entitled "Effects of Pretreatment with Phenobarbital and Chlorpromazine in the Acute Toxicity of Benzene in Rats" has been submitted to Toxicology and Applied Pharmacology for publication.

PUBLICATIONS

Drew, R. T., and Fouts, J. R.: Effects of pretreatment by phenobarbital and chlorpromazine on the acute toxicity of Benzene. Toxicol. Appl. Pharmacol. 24: 1972 (in press).

Serial No.: NIEHS-PT-005
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "A Comparative Study of the Mixed-Function Oxidase (MFO) Systems of Rabbit Lung and Liver with Special Emphasis on the Properties of the Pulmonary Mixed-Function Oxidase System"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., T. E. Eling, Ph.D., G. E. R. Hook, Ph.D., F. Law, Ph.D., and R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., G. Foureman, B.S., L. Gilmore, B.S., and R. Simmons, B.S.

COOPERATING UNITS: Biometry Branch, Animal Science and Technology Branch

MAN YEARS:

Total:	1.0
Professional:	0.6
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this investigation are (1) to study the effect of known inducers or inhibitors (including environmental agents) of the hepatic MFO system on pulmonary MFO activity, (2) to compare in vitro pulmonary microsomal drug metabolizing activity using several substrates, with the activity of the isolated, perfused lung preparation, and (3) to identify the cell type or types in rabbit lung which contain the MFO activity.

METHODS EMPLOYED: Standard differential centrifugation, spectrophotometric, radio-chemical and chromatographic procedures are utilized.

MAJOR FINDINGS: Pretreatment of rabbits with 3-methylcholanthrene (3-MC) (20 mg/kg, i.p.) caused an increase in the level of liver cytochrome P450 (about double) and in lung cytochrome content (about 50%) when the animals were treated 3 days prior to sacrifice. The liver cytochrome, in the reduced, CO-bound form, had its absorption maximum at 448 nm.

In spite of the increases in liver and lung microsomal cytochrome levels, there was no increase in benzpyrene hydroxylase specific activity in either tissue. This has been observed before in rabbit liver.

Rabbits were given high oral doses of several commercial polychlorinated biphenyls (300 mg per week for 14 weeks; Aroclors 1221, 1242 and 1254). The animals were sacrificed 1 week after the last dose of Aroclor. In lung microsomes from female rabbits dosed with Aroclor 1242 or 1254, biphenyl 4-hydroxylase, benzphetamine N-demethylase and benzpyrene hydroxylase specific activities were significantly lower than in control animals. Although these activities appeared to be lower in pulmonary microsomes of Aroclor 1221-treated female rabbits than in controls, the differences were not statistically significant. In liver microsomes from these same animals, benzphetamine demethylase activity was not altered by any of the Aroclors. However, there was a significant induction of hepatic microsomal biphenyl hydroxylase specific activity in Aroclor 1254-treated rabbits, and an apparent but statistically insignificant increase of this activity in Aroclor 1221- and 1242-treated animals. Benzpyrene hydroxylase activity tended to be inhibited in liver microsomes of Aroclor-treated animals but this effect was also not statistically significant.

Although chlorcyclizine is converted to metabolites by rabbit liver microsomal preparations and whole homogenate in vitro, no metabolism was noted with lung microsomes or whole homogenate. Imipramine, methadone, pentobarbital and aniline were all metabolized by both lung and liver microsomes and the metabolic profiles were, in general, similar. Liver whole homogenate that was not fortified by the addition of a TPNH generating system retained the ability to metabolize all compounds tested, while only methadone was metabolized by unfortified lung homogenate. Methadone is also metabolized by the isolated, perfused lung preparation but the blood contains a methadone metabolite that could not be detected (extracted from) the perfused lung tissue after homogenization.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental pollutants that are inhaled come directly into contact with the lungs of humans and other mammals. The ability of the lung to metabolize foreign organic compounds and the properties of the enzyme system involved in this metabolism are of interest. Also important are the potential effects of inducers or inhibitors of lung and liver MFO systems on an individual's in vivo ability to toxify and detoxify xenobiotics or synthesize and metabolize endogenous substrates such as steroids and phospholipids.

PROPOSED COURSE: The induction of rabbit liver and lung microsomal cytochrome levels by 3-MC without a corresponding increase in benzpyrene hydroxylase activity is an interesting observation. Other inducers of this type, including β-naphthoflavone and benzpyrene, will be tested to see if this property is general for "type II" inducers or whether it is peculiar for 3-MC in the rabbit. Further studies will be carried out using "solubilized" hepatic cytochrome, reductase and phospholipid fractions from control and 3-MC-induced rabbits to test the substrate specificity of rabbit cytochromes P450 and P448 towards benzpyrene and benzphetamine metabolism.

Studies of drug and chemical metabolism in the isolated, perfused lung preparation will be continued and correlated with in vitro studies using liver and lung microsomes or whole homogenates.

PUBLICATIONS

Hook, G. E. R., Bend, J. R., Hoel, D., Fouts, J. R., Gram, T. E.: Preparation of lung microsomes and a comparison of the distribution of enzymes between subcellular fractions of rabbit lung and liver. J. Pharmacol. Exptl. Ther. 182: 474-490, 1972.

Bend, J. R., Hook, G. E. R., Easterling, R. E., Gram, T. E., Fouts, J. R.: A comparative study of the hepatic and pulmonary microsomal mixed-function oxidase systems in the rabbit. J. Pharmacol. Exptl. Ther. 183: 206-217, 1972.

Hook, G. E. R., Bend, J. R., Fouts, J. R.: Mixed-function oxidases and the alveolar macrophage. Biochem. Pharmacol. 21: 3267-3277, 1972.

Bend, J. R., Hook, G. E. R., Gram. T. E.: Characterization of lung microsomes as related to drug metabolism. Drug Metabolism and Disposition. 1: 1973 (in press).

Serial No.: NIEHS-PT-013
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Biosynthesis and Degradation of Lung Surfactant Phospholipids"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: D. Howell, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	1.3
Professional:	0.8
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The surfactant system of the lung has been shown to be important in maintaining alveolar stability. An important component in this system for rendering low surface-tension at the lung air-tissue interface is believed to be dipalmitoylphosphatidylcholine. The secretion of this phospholipid to the acellular lining of the lung is thought to occur in the form of the concentric lamellar organelles (CLO), which are about 1-2 microns in diameter and occur exclusively in the lung in type II cells. The objectives of this project are to develop a method for the isolation of these organelles from rabbits and to investigate their composition and metabolic properties.

METHODS EMPLOYED: Chromatography (TLC and GLC), liquid scintillation, density-gradient centrifugation and acrylamide gel electrophoresis.

MAJOR FINDINGS: A method was developed whereby the CLO from rabbit lungs could be isolated relatively pure and intact. These structures had high amounts of phosphatidylcholine and phosphatidylethanolamine with only trace amounts of neutral lipid. Palmitic acid was the major fatty acid found in these lipids. A phospholipid:protein ratio of approximately 12:1 was obtained repeatedly and served as a criterion of organelle purification. Acrylamide gel electrophoresis of one detergent-solubilized CLO gave a prominent, staining band migrating with albumin. Acid hydrolases, similar to those observed in lysosomes, were present in the CLO. High specific activities were observed for acid phosphatase and β-N-acetylglucosaminidase. Multiple forms of the latter enzyme appeared in acrylamide gels.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the participation of the CLO in the surfactant system of the lung would help to clarify whether or not these structures are important as a rate-limiting step in the elaboration of this system. A number of gases and drugs have been reported to modify drastically the number of CLO present in the type II cells. In infant respiratory distress syndrome, the CLO are essentially evacuolated while the lung is in atelectasis and contains decreased surfactant phospholipids. This suggests a correlation between structure and function for CLO.

The secretion of CLO hydrolases along with surfactant might be important in the regulation of levels of materials lining the distal passages of the lung or possibly in exerting bacteriostatic action important in pulmonary defense.

PROPOSED COURSE:

1. Examine the effects of various agents on the formation of the lung CLO.

2. Compare the uptake and decay of ^{32}p-orthophosphate in the CLO with that of surfactant phospholipids.

Serial No.: NIEHS-PT-015
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Extrahepatic Conjugating Pathways and the Effects of Environmental Contaminants and Other Xenobiotics on the Enzymes Involved"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., and M. O. James, Ph.D.

OTHER INVESTIGATORS: R. T. Drew, Ph.D., J. R. Fouts, Ph.D., C. Harper, Ph.D., G. E. R. Hook, Ph.D., G. Foureman, B.S., and L. Gilmore, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	2.2
Professional:	1.5
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are (1) the characterization of some microsomal and soluble conjugating and hydrating enzymes of extrahepatic tissues as compared to the liver enzymes, (2) to determine the effects of selected xenobiotics upon these enzymes, (3) to assess the relative importance in vivo of this extrahepatic enzyme activity as compared to the liver, following ingestion or administration of chemicals and drugs (substrates) via various routes including inhalation, and (4) to study the combined effects of several compounds (which have as a common step in their metabolism a glutathione-dependent reaction) on in vivo toxicity and on extraheaptic tissue necrosis by these chemicals.

METHODS EMPLOYED: Differential centrifugation, spectroscopic, fluorometric and radiochemical analysis.

MAJOR FINDINGS: Glutathione (GSH)-S-aryltransferase activity, with 1,2-dichloro-4-nitrobenzene as substrate, is widely distributed in the rabbit. Although the liver microsomal supernatant possesses the highest specific activity of any tissue, gut, lung, kidney, brain, spleen, and heart all contained significant levels of this enzyme. There was no activity in lysed whole blood or plasma. There was a gradual decrease in the transferase activity of homogenates

of the mucosa of the small intestine as one moved towards the distal end of the intestine.

Several individual polychlorinated biphenyl (PCB) isomers including 2,4,2',4'-2,3,4,5-, 3,4,3',4'-, and 2,6,2',6'-tetrachlorobiphenyls and 2,4,5,2',5'-pentochlorobiphenyl did not significantly affect GSH-S-aryltransferase activity of rabbit liver or lung soluble fractions. Similarly, pretreatment of rabbits with several commercial PCB's (Aroclors 1221, 1242, or 1254) had no significant effect on lung GSH-S-aryltransferase activity.

A radiochemical assay is being developed for GSH-S-epoxidetransferase activity using ^{14}C-styrene oxide as substrate. Initial experiments indicate that the only water soluble product formed in rabbit liver incubations is the GSH derivative of styrene oxide.

Microsomal epoxide hydrase activity of rabbit liver, lung, kidney and small intestine (proximal 12 inches) is being studied with ^{14}C-styrene oxide as a model substrate, chosen since its non-enzymatic rate of hydration is relatively slow. Intestinal microsomes had almost 50 percent the specific activity of hepatic microsomes and kidney microsomes were about 10 percent as active as those from liver. Lung microsomes convert substrate to styrene glycol at approximately double the non-enzymatic rate (no protein present), but it is still uncertain whether this is a true enzymatic effect or a non-specific interaction with protein.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The conjugation of foreign organic compounds and their oxidative metabolites by the lung and gut, as well as other extrahepatic tissues, may play a significant role in the overall toxicity of agents which enter the body via the pulmonary system or via the alimentary canal. The fate of any active metabolites of microsomal metabolism such as epoxides and N-oxides formed in extrahaptic tissues may be important with regard to localized tissue necrosis.

PROPOSED COURSE: Characterization of GSH-S-epoxidetransferase and epoxide hydrase activities of rabbit extrahepatic tissues will be continued and the relative importance of these two competitive pathways will be assessed by determining the relative amounts of glycol and GSH derivative formed using 10,000 g supernatant fractions of the tissues involved. The effect of administration of substrates for GSH-S-aryl- and S-epoxidetransferase activities upon GSH levels in various tissues will also be studied. The effect of known glutathione depletors, including those that are active *per se* (e.g., diethyl maleate) and those which must be metabolized by the microsomal system (e.g., carbaryl) upon the toxicity of organophosphate esters and upon the degree of necrosis in extrahepatic tissues following chlorobenzene or bromobenzene, will also be investigated.

Serial No.: NIEHS-PT-016
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Microsomal Mixed-Function Oxidase System from Rabbit Lung and Liver: Solubilization, Isolation, Reconstitution and Specificity"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: T. C. Orton, Ph.D., and R. Simmons, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	2.25
Professional:	1.25
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The microsomal mixed-function oxidase (MFO) system of mammals exhibits species, sex, and tissue variations. Treatment of animals with various pharmaceutical agents, environmental pollutants, and other exogenous compounds can bring about changes in the MFO system(s). Both stimulation and inhibition of these systems can be elicited, and in some cases this can be accomplished with the same compound. It is not understood if these changes are due to direct effects on the major components of the MFO, to alterations in the membrane environment, or to changes in the concentrations or kinds of endogenous compounds that interact with the MFO. A better understanding of this problem depends on the purification and reconstitution of the various components of the system from animals and tissues that exhibit differences in their mixed-function oxidase specificities. The primary objectives of this project are:

1. Solubilization of the cytochrome P450, TPNH-cytochrome c reductase, and lipid components of the MFO system from the liver and lung of treated and control rabbits.

2. Reconstitution of MFO activity from these isolated and purified components.

3. Characterization of cytochrome and reductase fractions with respect to molecular weight and degree of purity.

METHODS EMPLOYED: Cytochrome P450 is solubilized via sonication and detergent treatment and fractionated out with ammonium sulfate precipitation. The reductase is obtained from acetone extracted microsomes. Lipid fractions are eluted from a DEAE cellulose column onto which solubilized microsomes have been placed. Most drug substrate metabolisms and bindings are assayed by standard procedures with the exception of testosterone metabolism which is being analyzed via TLC utilizing newly developed solvent systems for the separation of the testosterone metabolites. Parathion metabolism is assayed using ^{14}C ring-labeled parathion and TLC.

Molecular weight estimations are being made on Sepharose 4B utilizing suitable enzyme markers. Contamination of the various fractions by other chromophores is being investigated spectrophotometrically.

MAJOR FINDINGS: Both the cytochrome P450 and TPNH-cytochrome c reductase fractions contain significant amounts of cytochrome b_5 but little or no DPNH b_5 reductase. Preliminary experiments indicate that these fractions have molecular weights well in excess of typical values reported for other cytochromes and reductases that have been purified. Single peak elution profiles for these fractions suggests that they exist in the form of complex units rather than as heterogeneous protein solutions.

Cytochrome P448 from 3-methylcholanthrene-treated animals has been found to be more active in the conversion of parathion to paraoxon than P450 from control or phenobarbital-treated animals. The enzyme activity of reconstituted systems containing these cytochrome fractions is now being investigated with other substrates. A new method for quantification of testosterone metabolites has been developed and the microsomal metabolism of this compound in rabbit lung and liver has been characterized.

The cytochrome P450 fraction from rabbit lung has been solubilized by sonication, detergent treatment and ammonium sulfate fractionation of lung microsomes. This fraction is highly contaminated with TPNH cytochrome c reductase. Attempts are now underway to eliminate this contamination so that the substrate specificity of this lung cytochrome can be investigated at less than saturating reductase concentrations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of mixed-function oxidase systems is required if drug interaction, drug metabolism, disposition of ingested or inhaled environmental pollutants, and pesticide detoxification are to be fully comprehended. This requirement makes it essential that investigations be carried out under conditions which allow the obligatory components of the system to be independently manipulated. With isolated, solubilized mixed-function oxidase systems the questions of specificity, mechanisms and kinetics can be approached in a more precise manner than that afforded through the use of microsomal preparations.

PROPOSED COURSE: The present work involving the solubilization, reconstitution, and substrate specificity of the MFO system from rabbit liver and lung will be

continued. Attempts to eliminate the impurities of the various fractions and characterize the cytochrome and reductase will be emphasized.

PUBLICATIONS

Orton, T. C., Philpot, R. M., and Fouts, J. R.: Testosterone hydroxylation in rabbit liver and lung microsomes. Federation Procs., Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 1973.

Serial No.: NIEHS-PT-017
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Chlorinated Environmental Contaminants: Sites of Storage, Uptake, Distribution and Excretion"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., and R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: R. S. Chhabra, Ph.D., T. E. Eling, Ph.D., J. R. Fouts, Ph.D., G. E. R. Hook, Ph.D., R. Easterling, G. Foureman, B.S., L. Gilmore, B.S., D. L. Howell, B.S., and C. Williams, M.S.

COOPERATING UNITS: Animal Science and Technology Branch

MAN YEARS:

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The polychlorinated biphenyls (PCB) and some chlorinated insecticides posses high chemical stability and high lipid solubility. As a result of these properties they tend to be widely spread throughout the environment and to become persistant tissue residues, particularly in fatty tissues. The objectives of this study are (1) to study the pharmacodynamics and subcellular site(s) of PCB storage in rat liver and lung using ^{14}C-2,4,5,2',5'-pentochlorobiphenyl (^{14}C-PCB) as a model compound, (2) to determine the rate of uptake from blood and disappearance of radioactivity from several rat tissues following single, tracer doses (i.v.) of ^{14}C-PCB, and (3) to determine the rate and routes of excretion of radioactivity after single oral and parenteral doses of ^{14}C-PCB.

METHODS EMPLOYED: The floating fat layer (FFL) is isolated from liver or lung by homogenization and centrifugation. The PCB content in the FFL from ^{14}C-PCB pretreated animals is compared to aliquots of whole homogenate from the same rat. Radioactivity of homogenate, lipid particles and feces is generally determined after tissue oxidation, because this overcomes the problem of incomplete extraction, prior to liquid scintillation counting.

MAJOR FINDINGS:

1. Preliminary results indicated that lipid droplets from liver contained about 25 percent of the total PCB in liver. This suggested a storage function for these particles which might be important. We were concerned about redistribution during homogenization and further work indicated we cannot rule this out.

2. The maximum specific activity in tissues (dpm/g tissue) following a single, i.v. dose of ^{14}C-PCB (0.06 mg/kg) occurred within 1 to 2 hours in most organs - e.g. - liver, lung, heart, brain, testes, spleen, and kidney, although the maximum specific activity in fat occurred 28 hours after treatment. The liver contained by far the greatest percentage of administered radioactivity, followed in order by lung, kidney, heart, brain, and testes. Much less activity was taken up into liver and lung following oral administration over the time period of 1 to 28 hours, suggesting that intestinal absorption may be incomplete (similar results were obtained in fish). One week after single oral or i.v. doses of ^{14}C-PCB the specific activity of lung was greater than for liver, although fat contained higher levels of activity than either liver or lung at these later times.

3. Urinary excretion of radioactivity over a one week period after oral (0.23 mg/kg) or parenteral (0.06 mg/kg) doses of ^{14}C-PCB was minimal (1% and 5%), with most of the activity occurring in the 0-24-hour urine samples in each case. Fecal excretion was extensive in both cases accounting for approximately 80 percent of the orally closed radioactivity and about 60 percent of the parenterally-dosed activity.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because PCB's occur widely in nature and are concentrated in lipid residues via the food chain, particularly in marine species, the storage and elimination of these compounds in animals and man is of direct importance to environmental health. In addition, it may be important to investigate potential interactions of PCB's with other chemicals including drugs, food additives or pesticides from the aspect both of the competition for a common storage site and from the aspect of enhanced biological effects (increased toxicity).

PROPOSED COURSE: Hopefully, the subcellular storage site (e.g. - role of lipid droplets) for ^{14}C-PCB in liver and lung can be most clearly defined by the use of autoradiography. In addition, the half-life of ^{14}C-PCB depletion in liver, lung and fat of the rat will be determined along with plasma decay curves following i.v. administration. The nature of the radioactivity present in liver, lung and fat (i.e., unchanged 2,4,5,2',5'-pentochlorobiphenyl or metabolite) will also be determined at a few time points.

Serial No.: NIEHS-PT-020
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Sulfur Dioxide: Characterization and Modification of the Metabolic Pathway"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: H. J. Cohen, M.D., Ph.D.

OTHER INVESTIGATORS: K. V. Rajagopalan and J. Johnson, Department of Biochemistry, Duke University

COOPERATING UNITS: Department of Biochemistry, Duke University, Durham, North Carolina

MAN YEARS:

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: This is an effort to understand and possibly manipulate one mechanism by which animals detoxify a common air pollutant, sulfur dioxide. Studies seek to characterize the enzyme, sulfite oxidase, which metabolizes sulfur dioxide to sulfate, and to determine what factors, both environmental and experimental, serve to control the activities of this enzyme system.

METHODS EMPLOYED: Most studies on the enzyme are performed using visual spectrophotometry to visualize the heme group and using electron paramagnetic resonance (EPR) spectroscopy (in conjunction with Dr. K. V. Rajagopalan) to visualize the molybdenum prosthetic group. Gel electrophoresis has been employed using heme-specific and enzyme activity-specific stains. Nutritional studies employing diets containing tungsten and vanadium have served to elucidate mechanisms of control of the enzyme activity. Immunologic techniques including antibodies against sulfite oxidase have been helpful in correlating functional and structural parameters.

MAJOR FINDINGS: Sulfite oxidase has been purified to homogeneity from rat liver and found to contain two atoms of molybdenum and two molecules of heme per molecule of enzyme. The purified enzyme has a sulfite-dependent molybdenum EPR signal the pH-dependency of which distinguishes it from all other similar EPR signals examined to date. This signal is observed in liver slices, liver homogenates and intact mitochondria and the magnitude correlates with the

amount of enzyme present. The developmental pattern of the enzyme has been compared with other enzymes and found to be quite distinctive, increasing by a factor of 15 from birth to 3 weeks and then remaining essentially level. Tungsten when put in the drinking water of rats at levels of 1 to 100 parts per million produces a dose-dependent loss of both hepatic sulfite oxidase and xanthine oxidase activity and a concomitant loss of hepatic molybdenum and EPR-detectable molybdenum. Studies using antibodies to sulfite oxidase have shown that cross-reacting material without enzyme activity is still present in tungsten-treated rats and this material is localized in the same intracellular compartment as in control rats. This material contains the same b_5-like cytochrome as the native enzyme and the heme is precipitated by the anti-sulfite oxidase antibody.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the mechanisms by which the body detoxifies sulfur dioxide and the factors which control these metabolisms may explain why certain individuals or groups are more sensitive to the deleterious effects of SO_2 and how we can alter the sensitivity to this noxious agent.

PROPOSED COURSE:

1. Purify the protein made in the presence of tungsten and characterize it by physical and chemical means especially as to tungsten, molybdenum and heme content and molecular weight.

2. Using exposure chambers, determine if the acute toxicity to inhaled sulfur dioxide, i.e., LC_{50}, is affected by the loss of this enzyme due to tungsten treatments. Do similar study with intraperitoneal sulfite to see if correlation exists between the two agents (SO_2 and SO_3^-).

3. Determine if decreased level of sulfite oxidase produces a change in urinary sulfur metabolites.

4. Compare oral and injected tungsten on liver, kidney and intestinal activity of sulfite oxidase.

5. Make antibodies to xanthine oxidase and show if a similar effect by tungsten-feeding occurs to this enzyme as was seen with sulfite oxidase.

PUBLICATIONS

Cohen, H. J., Betcher-Lange, S., Kessler, D. L., and Rajagopalan, K. V.: Hepatic sulfite oxidase: Congruency in mitochondria of prosthetic groups and activity. J. Biol. Chem., 247: 7759 (1972).

Cohen, H. J.: The use of diaminobenzidine for spectrophotometric and acrylamide gel detection of sulfite oxidase and its applicability to hydrogen peroxide generating enzymes. Anal. Biochem. (in press).

Serial No.: NIEHS-PT-021
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Lung Membrane-Bound Nicotinamide Adenine Dinucleotide Glycohydrolase"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: S. Gipson, B.S., and D. Howell, B.S.

COOPERATINING UNITS: None

MAN YEARS:

Total:	0.7
Professional:	0.2
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: Nicotinamide adenine dinucleotide glycohydrolase (E.C. 3,2,2,5), or NADase, cleaves the nicotinamide-ribose link of NAD and was found, in this laboratory, to be very active in rat lung tissue. Others have shown this enzyme to greatly increase in lungs infected with M. tuberculosis. The enzyme can act also as a transglycosidase and incorporate such drugs as isoniazid into NAD by exchange with nicotinamide. The objectives of the current program are to study the properties and subcellular distribution of this enzyme and to examine whether or not the transglycosidase activity observed in vitro is a real process in vivo.

METHODS EMPLOYED: UV spectroscopy, pH stat, column chromatography, liquid scintillation and paper chromatography were employed.

MAJOR FINDINGS: Lung NADase occurs exclusively in membrane fractions of the rat lung. Highest specific activities were observed in the 25,000 g and 105,000 g sediments. The membrane-bound enzyme had a broad pH optimum between 5.9 and 6.9 and was "insensitive" to isoniazid. Nicotinamide was a relatively potent inhibitor of glycohydrolase and transglycosidase activity. Lungs removed from rats injected with ^{14}C-isoniazid were shown to contain an isolatable isoniazid analog of NAD. The decay rate of this analog in lung was approximately twice that observed for whole tissue ^{14}C.

Preliminary studies of solubilized NADase, obtained from a lipase-digested membrane fraction of lung, revealed that at least two enzyme forms exist in

the lung which could be readily separated by carboxymethyl-cellulose column chromatography.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The delocalization of lung NAD glycohydrolase has been postulated by other workers as a process which might initiate cellular degenerative processes. Understanding the type of union of this enzyme with membranes might establish a suitable test system for this delocalization process conferred by micro-organisms or environmental pollutants and also explain why certain phagocytic cells, like the alveolar macrophage, do not have NADase.

An investigation of lung tissue levels and extent of retention of the isoniazid analog of NAD could bring insight as to whether or not this process is important in the mechanism of action or toxic responses observed for this anti-tuberculosis drug. By this NADase activity other drugs and chemicals might be incorporated into other metabolic intermediates and, thereby, this process might serve as an *in vivo* prototype of certain covalent reactions of xenobiotics. In a broad sense, proof of such reactions *in vivo* could explain many toxic and autoimmune reactions observed with chemicals in mammals and allow development of *in vitro* systems to test this potential property of compounds.

PROPOSED COURSE:

1. Continued investigation of the multiple forms of NADase and the nature of their binding to membranes.

2. Comparison of decay rates of the ^{14}C-isoniazid analog of NAD and ^{14}C-nicotinamide -NAD in the rat lung *in vivo*.

PUBLICATIONS

DiAugustine, R. P., Gipson, S., and Howell, D.: Lung NAD glycohydrolase. Formation of INH analog of NAD *in vitro* and *in vivo*. Federation Procs., Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 1973 (in press).

Serial No.: NIEHS-PT-022
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Prostaglandin Biosynthesis and Metabolism by Lungs. What are the Factors Controlling their Biosynthesis and Metabolism and What is Their Relationship to Physiological Function. Effect of Pollutants on This System"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: T. E. Eling, Ph.D.

OTHER INVESTIGATOR: D. G. Parkes, B.S.

COOPERATING UNITS: E. O. Oswald, Ph.D., and R. T. Drew, Ph.D.

MAN YEARS:

Total:	1.0
Professional:	0.3
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: This project is directed toward obtaining a better understanding of lung function. The primary objective is to investigate the biosynthesis and metabolism of prostaglandins by the lung and to relate changes in steady-state concentrations of the various prostaglandins to biological effects. In addition, the effects of various environmental agents (SO_2, O_3, NO_2, O_2, etc.) on the ability of the lung to synthesize and degrade prostaglandins will be investigated. An attempt will be made to correlate the biological effect of pollutants and modulation of prostaglandin levels.

METHODS EMPLOYED: Prostaglandins are analyzed by a combination of quantitative thin-layer chromatography and liquid scintillation counting. The radioactive prostaglandins, produced by lung tissue from the precursor ^{14}C-arachidonic acid, or the non-metabolized H^3-prostaglandins are extracted from the incubation medium, and separated on thin layer plates. The radioactive areas are measured by liquid scintillation techniques. Guinea pig lung serves as an enzyme and co-factor source for both biosynthesis and metabolism. Further identification of the prostaglandins uses a combination of thin-layer chromatography, continuous gradient elution column chromatography, and chemical derivatization followed by gas chromatography and mass spectrometry.

MAJOR FINDINGS: Prostaglandin (PG) synthetase activity in guinea pig lung is located primarily in vitro in the microsomal fraction. A method was developed, using ^{14}C-arachidonic acid as the precursor, to separate and quantify the formed-PG. This assay was optimized in vitro with respect to protein, time, pH, substrate and co-factor. Guinea pig lungs in vitro form $PGF_{2\alpha}$, traces of PGE_2 and an unknown material (PGD). The identity of PGE_2 and $PGF_{2\alpha}$ was established using a variety of chemical techniques. The unknown material could not be identified; analysis (GLC, mass spect.) was complicated by the inability to obtain a pure fraction of the unknown material, and the lack of authentic PG derivatives as standards. Several of the commonly employed derivatization methods were found to dehydrate the 15-hydroxy group, which leads to a misassignment of mass-spectral peak and thus could lead to errors in structure determination.

A heat-stable co-factor necessary for the formation in vitro of $PGF_{2\alpha}$ was found in the soluble fraction. Various compounds were found to alter the formation in vitro of $PGF_{2\alpha}$ and the unknown compound (PGD). The amines 5-hydroxytryptamine (5-HT) and norepinephrine (NE) were potential inducers of PG biosynthesis in vitro; 5-HT increased $PGF_{2\alpha}$ while NE increased PGD. Glutathione, histamine, and hydroquinone increased biosynthesis in vitro of both $PGF_{2\alpha}$ and PGD. The effect of 5-HT on $PGF_{2\alpha}$ formation in vitro occurred only over a narrow concentration range. These compounds affect only the Vmax but not the Km for the biosynthesis. Indomethacin was found to be a potential inhibitor of biosynthesis in vitro.

PG metabolizing activity in vitro was found in the soluble fraction of guinea pig lung. An in vitro assay, using ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ as substrates, for metabolism was developed and optimized.

The effect of environmental agents on the rates of biosynthesis and metabolism in vitro was investigated. Exposure of guinea pig to various concentrations of SO_2 for various times, did not alter either PG biosynthesis or metabolism as measured in vitro in preparations from these exposed animals. Exposure of guinea pigs to 100 percent O_2 for 48 hours did not alter biosynthesis, lung weight, or lung total protein, but did depress PG metabolism (60%) by lung fractions from these animals. Metabolism of PG in vitro by lung fractions was depressed by 100 percent O_2 exposure of animals in a time-dependent manner and reached 90 percent inhibition after 72 hours of O_2 exposure. The effects of animal exposure to O_2 appears to be specific because other soluble lung enzymes were not depressed by these treatments of the animals.

Exposure of guinea pigs to 90 ppm of NO_2 for 6 hours also depressed PG metabolism by lung fractions in vitro, thus suggesting a general toxic effect of oxidants on the metabolizing enzyme system. The effect of O_3 exposure of guinea pigs on guinea pig lung prostaglandins biosynthesis and metabolism in vitro is currently being investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Prostaglandins represent a new class of endogenous substance which possess unique biological activity in lung. Prostaglandins of the E class are potent bronchial dilators, while prostaglandins of the F class are potent constrictors. Both classes of prostaglandins have been implicated in inflammatory responses.

Because these substances are not stored in most tissue, control of biosynthesis and metabolism is important to their physiological activity. Pathological changes or conditions produced in various disease states or in response to pollutants or environmental agents could involve an effect on either prostaglandin biosynthesis or metabolism. Understanding the mechanisms for the control of prostaglandin levels in lung tissues should contribute to the overall understanding of biological functions of these lipids.

Depression of the lung metabolizing system could be of direct mechanical importance because prostaglandins and their derivatives have been used as therapeutic agents. Thus interactions are possible and should be studied.

PROPOSED COURSE:

1. Investigate the effect of animal exposure to oxidants (O_3, NO_2) on the PG metabolizing system of guinea pig lung.

2. Establish time course and dose-response curves for the effect of oxidants on PG metabolism.

3. Study the mechanism of inhibition of metabolism. Determine if this inhibition is a specific effect related to oxidant toxicity to lung, or is a non-specific effect.

4. Determine the pathologic and/or physiologic effects of increased PG in lung.

5. Investigate effects of respiratory diseases or allergic conditions on biosynthesis and metabolism of PG in lung.

6. Perform comparative metabolism studies using isolated perfused lung preparations (comparison with microsomal systems).

7. Investigate PG metabolism and oxidant effects on this metabolism in other animal species.

Serial No.: NIEHS-PT-023
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Inhalation Toxicity of Compounds Common to Household Aerosols"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. T. Drew, Ph.D.

OTHER INVESTIGATORS: J. R. Lo, Ph.D., R. P. Roth, Ph.D., G. J. Taylor IV, M.D., E. Lores, B.S., F. Harrington, M. Riley, and J. Schroeder

COOPERATING UNITS: Animal Science and Technology Branch (Pathology and Histology), and Biometry Branch

MAN YEARS:

Total:	3.9
Professional:	2.1
Other:	1.8

PROJECT DESCRIPTION

OBJECTIVES: To determine acute and chronic inhalation toxicity of selected compounds common to household aerosols and to identify any potential synergistic interactions. Compounds currently under investigation include Rehydrol ASC (an aluminum-chloride-hydroxide-propylene glycol complex used in aerosol deodorants), "Airwick," and fluorocarbon 12 (Freons).

METHODS EMPLOYED:

1. Exposure techniques: Aerosols of Rehydrol ASC are generated from an aqueous solution with a compressed air-driven nebulizer. Airwick vapors are generated by passing the chamber intake air through a box having a large surface area saturated with liquid airwick. Fluorocarbon 12 concentrations are obtained by blending pressurized fluorocarbon 12 gas and air in the appropriate ratio. Exposures are always carried out under dynamic conditions with contaminant concentrations being monitored frequently during exposures.

2. Evaluation to toxicity: Several techniques are utilized to determine the effects of exposures. These include histologic preparation of tissue and microscopic examination for pathological or morphological changes; investigation of the mixed-function oxidase (MFO) activities of both lung and liver microsomes after animal exposure; assessment of weight gain and

organ weights after exposure; investigation of any change in respiratory physiological parameters such as breathing rate and volume, resistance, and static and dynamic compliance; measurement of electrocardiographic and hemodynamic parameters, and myocardial contractility changes after and during exposures; and measurement of deposition and clearance of chemicals in the lung, and body distribution and elimination of chemicals after exposure.

MAJOR FINDINGS:

1. Rehydrol ASC: Exposure to this compound for 3 days causes an increase in lung weights in both rabbits and hamsters. In hamsters the minimum dose to cause this lung weight increase was 35 mg/M^3 for 3 successive days. The increase in lung weight is highest at 4 days and is gone a week later. During this time, the aluminum does not leave the lung. The MFO specific activities are diminished after such an exposure, but this appears to be a dilution effect rather than an inactivation of microsomal enzymes in both rabbits and hamsters. Exposure of hamsters to 50 mg/M^3 for 6 weeks induces a granulomatous lesion at the peripheral bronchiole which is still present at 2 weeks and has not regressed at 12 weeks after last exposure to Rehydrol aerosols.

2. In a series of experiments designed to confirm work done elsewhere, rabbits were exposed to airwick vapors. After exposures, MFO activities were measured in both lung and liver microsomes. Rats were also injected, i.p., with various constituents of airwick and subsequently used in hexobarbital sleeping time experiments. Thus far, none of the treatment protocols has elicited a demonstratable effect of airwick exposure on MFO activities or duration of drug (hexobarbital) action in rats.

3. Freon: Acute exposure of rabbits to dichlorodifluoromethane (fluorocarbon 12) results in a dose-related depression in left ventricular conduction and a left shift of the force-velocity curve, both considered indices of myocardial contractility depression. This substantiates previous findings in cats.

No changes in arterial oxygen tension, CO_2 tension or pH accompanied the myocardial contractility depression.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of the aerosol "spray can" dispenser has resulted in a very complex and wide variety of materials being available in respirable form. While the acute toxicity of many of the "active ingredients" in such dispensers has been investigated, little is known about the chronic toxicity of these compounds or the toxicity of combinations of these compounds and "inert ingredients" of the pressurized cans. Elucidation of the chronic toxicity of these compounds and their potential combinations can be of vital importance to environmental health.

PROPOSED COURSE: The studies on Rehydrol ASC and Freons will continue. The Rehydrol ASC project will concentrate on the histologic lesion and the clearance of aluminum from the lung after exposures for varying times. The Freon project will continue to assess cardiovascular effects of inhaled Freons as well as

studies of interactions of Freons with other chemicals - e.g. - do Freons affect lung uptake or storage or actions of other chemicals?

Serial No.: NIEHS-PT-024
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Influence of Inhaled Environmental Agents on Mammalian Pulmonary Mechanics"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: R. P. Roth, Ph.D., and R. T. Drew, Ph.D.

OTHER INVESTIGATORS: T. E. Eling, Ph.D., R. D. Pickett, Ph.D., G. J. Taylor IV, M.D., Frank Harrington, Mike Riley, and Fred Suddreth

COOPERATING UNITS: Animal Science and Technology Branch

MAN YEARS:

Total:	0.8
Professional:	0.5
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: Pulmonary function studies will be conducted utilizing standard and newly devised physiological techniques to assess the effects of chronic and acute inhalation exposures of irritants, oxidants, solvents, and propellants on the intact rabbit. These findings will be correlated with data from cardiovascular, biochemical and histological investigations. The respiratory physiologic data derived from these studies will be compared with similar data derived from the isolated perfused rabbit lung to assess the usefulness of such a test model.

MAJOR FINDINGS: Early studies have concentrated on development of the methodology. Preliminary comparative results in terms of pulmonary resistance, dynamic compliance and time constants indicate reasonable agreement between *in vivo* and *in vitro* preparations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our approach to studies of effects of environmental chemicals on this organ system will be to use sensitive pulmonary function indices including frequency dependent compliance and possibly critical closing volume to provide early detection of pathologic processes in the small airways. The ability to measure subtle changes in respiratory physiologic parameters may provide us with

another tool to assess the effects of inhaled materials.

PROPOSED COURSE: This project will continue with emphasis in three general areas: (1) to assess the respiratory physiologic effects of single and multiple exposures to individual chemical compounds and combinations of compounds, (2) further characterization of normal physiologic parameters for comparison with *in vitro* studies, and (3) comparison studies involving responses to known pharmacologic agents.

Serial No.: NIEHS-PT-025
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Arylhydroxylases of Lung"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: C. Harper, Ph.D., R. T. Drew, Ph.D., and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., R. E. Easterling, L. B. Gilmore, B.A., and J. L. Schroeder

COOPERATING UNITS: Animal Science and Technology Branch (Histology and Electron Microscopy)

MAN YEARS:

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To investigate in detail the arylhydroxylases of lung using a number of simple aromatic compounds as substrates. Because some of these compounds (e.g., halogenated benzenes) are known to cause liver necrosis, their effects on the lung after inhalation will be investigated morphologically as well as enzymically.

METHODS EMPLOYED: Microsomes were isolated from lung. Arylhydroxylase activities are assayed using radiochemical methods. Morphological investigations involve both light and electron microscopic methods.

MAJOR FINDINGS: So far, results suggest that on a specific activity basis, lung microsomes have certain arylhydroxylase levels which are at least as high as in liver microsomes (benzene and biphenyl hydroxylase).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most of the substrates are either materials of environmental concern (e.g., benzpyrene and biphenyl) or are used commercially. Some of them are known to cause liver necrosis but their effects on lung are not known or extensively studied as yet.

PROPOSED COURSE: These investigations will be coupled with an investigation of the sulfokinases responsible for the sulphation of the products of arylhydroxylation.

Serial No.: NIEHS-PT-026
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Lung Mixed-Function Oxidase Systems"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-023

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: R. T. Drew, Ph.D., M. Fields, and M. Riley

COOPERATING UNITS: Aerotoxicology Section of the Pharmacology and Toxicology Branch

MAN YEARS:

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To study the mixed-function oxidase enzymes of mammalian lung and the effects of inhaled xenobiotics on these enzymes.

METHODS EMPLOYED: Ultracentrifugation, radio tracer, spectrophotometric and aerosol techniques have been used to prepare lung microsomes and to assay the effect of an aerosol of piperonyl butoxide on the mixed-function oxidase enzymes and microsomal cytochromes.

MAJOR FINDINGS: The mixed-function oxidase enzymes (MFO) of lungs are similar in many ways to the MFO enzymes of liver which have been studied extensively. *In vitro*, these enzymes are inhibited by insecticide synergists (I_{50} values are in the range of 10^{-5} M), the I_{50} values observed with lung microsomes are similar to those observed with preparations of liver microsomes. Also, lung MFO enzymes have been shown to metabolize an insecticide synergist, piperonyl butoxide, to metabolites which are chromatographically similar to those produced *in vitro* by liver MFO enzymes.

Rabbits exposed to an aerosol of piperonyl butoxide for 15 minutes (est. dose = 2 mg/kg) and held for various periods of time up to 72 hours showed a decreased MFO enzyme activity and decreased cytochrome P450 level in the lung and liver microsome preparations. The greatest inhibition in liver MFO activity (approximately 40%) was observed 12 hours after exposure whereas the greatest inhibition

of lung MFO activity (approximately 60%) did not occur until 36 hours after exposure. The liver MFO activity recovered and showed slight induction (approximately 20%) by 48 hours after exposure whereas the lung MFO activity of the exposed rabbits was still 20 percent less than that of the controls at the end of 72 hours. Microsomal cytochrome P450 levels followed a similar pattern except that lung showed approximately 20 percent induction by 48 hours after exposure.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pesticide synergists are widely used in aerosol formulations of insecticides which are used in the home as well as in dusts, sprays and impregnated container liners. If the mixed-function oxidase enzymes of the lungs serve to protect the lungs and consequently the individual from the effects of inhaled xenobiotics, inhibition of these enzymes by the contents of household aerosols and frequently used insecticide dusts and sprays could play a significant role in lowering the resistance of exposed individuals to the actions of many inhaled toxicants.

PROPOSED COURSE: The work has been discontinued.

PUBLICATIONS

Matthews, H. B., and Fishbein, L.: Mammalian toxicology of insecticide synergists. A preliminary report presented at the ACS Meetings, New York, August 1972.

Serial No.: NIEHS-PT-027
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Localization of Xenobiotic Metabolism in Lung Cell Types"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: G. E. R. Hook, Ph.D., and J. R. Bend, Ph.D.

OTHER INVESTIGATORS: J. Zinkl, Ph.D., F. Talley, and R. Easterling

COOPERATING UNITS: Animal Science and Technology Branch

MAN YEARS:

Total:	1.0
Professional:	0.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To establish the cellular localization of xenobiotic metabolisms in the lung. The lung has 41 cell types, some of which may be exceptionally rich or poor in xenobiotic metabolism. Such information may explain localized injury of inhaled chemicals.

METHODS EMPLOYED: Alveolar macrophages were isolated by lung lavage. Cells were washed and disrupted and subcellular fractions prepared for assay and morphologic examination. Key enzyme systems were studied including MFO's, cytochrome oxidase and cytochrome c reductase, glucuronyl and glutathione transferases, cytochromes.

MAJOR FINDINGS: Biphenyl-4-hydroxylase, benzpyrene hydroxylase and benzphetamine demethylase activity of alveolar macrophages were very low compared with "lung" microsomes (prepared from homogenates of whole lung). Cytochrome P450 was not detected in macrophages. Both NADPH and NADH-cytochrome c reductase were present in macrophages at about 50 percent of levels in lung microsomes. Both glucuronide and glutathione transferases were absent in macrophages. Glutathione transferases was present at appreciable levels in lung soluble fraction (about 50% of activity of liver soluble fraction). The macrophage is obviously not a hot bed of xenobiotic metabolism as we have measured it.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of the lung cells rich vs. poor in xenobiotic metabolizing systems may help understanding of localized lung damage by chemicals and the

role of metabolism in that damage. The lack of metabolizing systems in the macrophage raises the question of how this cell type deals with toxic chemicals engulfed by phagocytosis.

PROPOSED COURSE: Further studies of other metabolizing systems in macrophages (sulfokinases, N-oxidations, epoxidations) will be made. Response of macrophage systems to inducers of lung xenobiotic metabolism will be determined. Development of histochemical technics to identify other cell types with presence or absence of metabolizing systems will depend on cooperation with other units (ASTB - histology) or additional (histologically-trained) technicians.

PUBLICATIONS

Hook, G. E. R., Bend, J. R., and Fouts, J. R.: Mixed-function oxidases and the alveolar macrophase. Biochemical Pharmacology, 21: 3267-3277, 1972.

Bend, J. R., Hook, G. E. R., Fouts, J. R., and Gram, T. E.: Further studies of the microsomal mixed-function oxidase system of rabbit lung. Fifth International Congress on Pharmacology. Abstracts of Volunteer Papers, p. 18, 1972.

Serial No.: NIEHS-PT-028
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Enzyme Activities of the Lung Lining Layer"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: R. P. DiAugustine, Ph.D., and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: R. E. Easterling, L. B. Gilmore, B.A., and D. L. Howell, B.S.

COOPERATING UNITS: Animal Science and Technology Branch (Histology and Electron Microscopy)

MAN YEARS:

Total:	0.6
Professional:	0.2
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: Numerous chemical analyses of the acellular lung lining layer have been made and there is little doubt concerning the identity of the surfactant material obligatory for normal lung function. However, the acellular layer is also a barrier to the environment and consequently may possess numerous, as yet unrealized properties. We are attempting to show that the medium bathing the alveoli is not merely required for its surface-tension-lowering properties but that it is also an actively metabolizing milleu.

The objectives of this investigation are (1) to determine the enzymatic composition of the lung lining, (2) to determine the origin of these activities, and (3) to investigate compositional variations of the lining under the influence of drugs, disease and airborne pollutants.

METHODS EMPLOYED: The acellular lung lining is removed by lavage. The lavage is analyzed for cell, chemical and enzymic composition using microscopic, spectrophotometric, chromatographic and electrophonelic methods.

MAJOR FINDINGS: We have detected numerous enzyme activities in lung lavages. These activities appear to derive from three compartments which are related to (1) the acellular lining layer, (2) the capillary bed, and (3) cellular damage. Preliminary investigations suggest that these three sources of lavage components can be distinguished using appropriate marker enzymes. The presence of lysosomal

hydrolases in the lung lavages correlates with evidence for the selective secretion of these enzymes by Type II cells.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Enzymic components of the acellular lung lining may play important roles in its regulation as well as defense of the lung.

Of considerable importance are methods which can provide evidence of disease or damage to the human lung. If, as preliminary data suggests, three compartments can be distinguished as far as contributions to the lung lining are concerned, then it may be possible to assess to a certain extent the degree and type of damage which has occurred to the lung merely by assaying lavage fluids for enzymic activities. For example, enzymes associated with serum would tend to increase in the lavage in cases where damage has occurred to capillary walls. Such an enzymic assessment could serve in the diagnosis of respiratory disease using sputum or lavage samples obtained at the clinic.

PROPOSED COURSE: In addition to a study of the further functional properties of the acellular lining as mentioned above, we will investigate potential bacteriostasis conferred by the lung acellular lining fraction.

Serial No.: NIEHS-PT-029
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Studies on the Mechanisms of Accumulation of Drugs and Pollutants in the Lung: Investigation of the Transport of Substances from Pulmonary Circulation and Inhaled Air into Lung Tissue. Metabolism of Xenobiotics by the Isolated Perfused Lung. Development of the Isolated Perfused Lung to Examine the Effect of Pollutants on Respiratory Mechanics"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: T. E. Eling, Ph.D., T. C. Orton, Ph.D., R. D. Pickett, Ph.D., M. W. Anderson, Ph.D. (Biometry Branch), F. Law, Ph.D., J. R. Bend, Ph.D., and H. Mehendale, Ph.D.

OTHER INVESTIGATORS: R. P. Roth, Ph.D., and D. G. Parkes, B.S.

COOPERATING UNITS: Biometry Branch, Animal Science and Technology Branch (Histology, Electron Microscopy)

MAN YEARS:

Total:	4.0
Professional:	3.7
Other:	0.3

PROJECT DESCRIPTION

PART I: "Studies on the Mechanisms of Accumulation of Basic Drugs and Pollutants by the Lung"

OBJECTIVES: The principal objective is to study the uptake and accumulation of basic chemicals in the lung tissue. Is the uptake process for basic compounds a simple diffusion through membranes, a specific transport mechanism(s), or a combination of these? Does the subsequent accumulation consist of binding to tissue or an accumulation against a concentration gradient or a combination of these? Accumulation could produce toxic effects to the lung or could serve as a kind of detoxication (via storage) mechanism. An additional objective is to study the accumulation and uptake of endogenous substances such as 5-hydroxytryptamine, and to examine the interactions between accumulation of these endogenous substance and exogenous compounds.

METHODS EMPLOYED: The lung perfusion apparatus consists of an artificial thorax, a recirculating blood supply, and an air supply. The lungs are ventilated with 95% air - 5% CO_2 by applying an alternating negative pressure to the artificial thorax. The circulatory system was designed with a blood reservoir directly above the lungs to which the labeled chemicals were added and from which samples of blood were removed for analysis.

Metabolites of the compounds as found in the blood and lung are studied by thin-layer and paper chromatographic techniques. Viability of the perfused lung was confirmed by light and electron microscopy. Hematocrit and blood pH were also monitored over the usual 2-hour perfusion period.

MAJOR FINDINGS: Studies with a number of amines of varying pKa indicated that accumulation by the perfused lung was dependent to some extent on the pKa of the amine. Amines with pKa > 8.5 (imipramine, amphetamine, chlorcyclizine, methadone) accumulated in lung tissue to a much greater extent than compounds with pKa < 7 (imidazole, aniline, triazine herbicides). Accumulation of amines by the lung was rapid; after addition of a dose to the blood reservoir, a steady-state was obtained in approximately 5 minutes.

Analysis of the steady-state was used to study the mechanism(s) of amine accumulation by the lung. Accumulation of basic amines was dependent on the steady-state blood concentration of the amine, and was resolved into a saturable and a linear component of accumulation. Amines with pKa < 7 accumulated by a process linearly related to blood concentration; the process was probably one of diffusion. Inhibition studies showed that a number of basic amines (but not non-basic amines) inhibited the accumulation of amphetamine and imipramine. We infer from these inhibitor studies that these inhibitors would also accumulate in the lung. Kinetic analysis of the saturable component of uptake showed that desipramine competitively inhibited imipramine accumulation and that imipramine inhibited amphetamine accumulation in a mixed inhibitor (part competitive, part non-competitive) manner. The linear component of amine accumulation by the perfused lung was not just simple diffusion. Sensitivity of the uptake process to temperature reduction suggested the involvement of lung transport system(s) in amine accumulation. An amine stored (accumulated) in lung could be displaced by perfusing the lung with a second amine indicating the reversibility of the accumulation and the potential for toxic (sudden) displacement. Methadone was the only compound used in these investigations which was found to be metabolized by the isolated perfused rabbit lung. Inhibitor studies with a series of fluorinated amphetamines of differing pKa indicated the importance of protonation of the nitrogen in the accumulation. The herbicide paraquat did not appear to accumulate in lung tissue but did produce a time-dependent edema formation.

SIGNIFICANCE TO-BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The significance of this project can be divided into distinct areas:

1. Development of a method for the perfusion of an isolated perfused lung - this allows studies with the lung uncomplicated by other organ functions. This preparation is significant in the fact that it is perfused and ventilated similarly to an intact organ and thus correlation of *in vivo* and *in vitro* studies is more likely.

2. The isolated perfused lung affords a good procedure to examine more directly than in vivo the effect of various pollutants and drugs on lung functions. Physiological and biochemical parameters can be examined and correlated.

3. The lung can act as an important site for accumulation of xenobiotics and thus accumulation could result in detoxification or altered toxicity. Thus the toxicity of a compound for the lung may be related to its ability to accumulate in the lung. In addition, those xenobiotics which accumulate in the lung could effect the accumulation and storage of other exogenous compounds or of endogenous substances such as norepinephrine and 5-hydroxytryptamine. The elucidation of the mechanism of accumulation could afford a means for predicting possible toxic effects and interactions of one chemical with another.

PROPOSED COURSE:

1. To further examine, through initial velocity and efflux studies, the mechanism(s) involved in the uptake and accumulation of amines and other pollutants by lung.

2. To investigate in more detail the structural requirements of the non-nitrogenous portion of the molecule.

3. To examine the mechanism(s) for the uptake and accumulation of 5HT- and norepinephrine in lung tissue and study the interaction between storage and release of exogenous and endogenous substances.

4. To investigate the accumulation of the polychlorinated biphenyls by lung and relate this to whole animal pharmacokinetics models.

5. To investigate the processes by which inhaled chemicals are stored in or transferred to the circulation of the perfused lung.

PUBLICATIONS

Eling, T. E., Orton, T. C., Pickett, R. D., Anderson, M. W., and Fouts, J. R.: Xenobiotic accumulation in the isolated perfused rabbit lung. Federation Procs., Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 1973 (in press).

Orton, T. C., Anderson, M. W., Pickett, R. D., Eling, T. E., and Fouts, J. R.: Xenobiotic accumulation and metabolism by isolated perfused rabbit lungs. J. Pharmacol. exptl. Therap., 1973 (in press).

PART II: "Development of the Isolated Perfused Rabbit Lung and Its Use in the Study of the Pharmacological and Toxicological Effects of Environmental Agents, Administered via the Perfusate or by Inhalation, on Pulmonary Mechanics"

OBJECTIVES: 1. To establish the most suitable methods for use of the isolated perfused rabbit lung in pharmacokinetic, metabolic, haemodynamic and pulmonary mechanical studies.

2. To study the effects on respiratory mechanics and pulmonary

haemodynamics of environmental agents administered via the perfusate or by inhalation.

METHODS EMPLOYED: The apparatus and techniques are similar to those described in Part I except for the addition of a number of recording devices for the measurement of perfusate flow rates, transpulmonary pressure, airway flow rates and inspiratory and expiratory volumes. From the recorded parameters values can be calculated for certain mechanical properties of the lung, e.g., dynamic pulmonary compliance, airway resistance, time constant and total pulmonary vascular resistance. Perfusate characteristics are studied before, during and after perfusion, e.g., pH, pCO_2, pO_2, haematocrit, etc.

MAJOR FINDINGS: The basic isolated perfused lung system has been improved as a result of such studies. Perfusate flow rates that more closely approximate the physiological norm have been achieved by improvement of preparatory procedures.

Measurement of the haematocrit has enabled the calculation of predicted lung weight changes due to edema during perfusion. This has assisted in the interpretation of chemical and drug uptake results in lungs that have developed edema.

Permanent recordings of perfusion rate have enabled a more accurate assessment of viability of the lung during perfusion and the preliminary observation of vasomotor effects of added drugs. Preliminary studies of pulmonary mechanics suggest that the isolated perfused lung is not significantly different in this respect from that of the intact animal.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: To date, the isolated, blood-perfused rabbit lung has been used at NIEHS to study the uptake and accumulation of chemicals from the blood into the lung. With the advent of the Section on Pharmacokinetics within the Pharmacology and Toxicology Branch, it is anticipated that this preparation will be used more frequently. In order to be able to interpret the results of such experiments with any degree of reliability, it is essential to know the physiologic status of the lung at any given point in the perfusion process. Because the ultimate goal is to extrapolate results obtained in the isolated perfused lung to the intact animal, a comparison of the *in vitro* and *in vivo* situations is required. In this phase of the project, collaborative studies with Dr. Roland R. Roth are foreseen.

From time to time innovative techniques are devised for use in pharmacokinetic and metabolic studies using the isolated perfused lung. An example of such a technique is the use of an artifical ferfusate. The effects of such techniques on the isolated perfused lung must first be established and thus the lung function study group acts as a "service arm" to other investigators using the isolated perfused lung.

The responses of the isolated perfused rabbit lung will be characterized prior to a study of the effects of "environmental agents". A comparison of the effects of chemicals on the lung as obtained in the isolated perfused rabbit lung with those obtained in the intact animal will provide an indication of

extrapolative reliability and may also suggest possible mechanisms of action. Further collaboration with Dr. Roland P. Roth is anticipated in this phase of the project.

PROPOSED COURSE: The effects of varying perfusate composition, ventilating gas composition and other parameters such as transpulmonary and pulmonary arterial pressures on lung functions will be studied.

Using optimal conditions for viability, the responses of the preparation will be characterized with respect to standard pharmacological agents in order that the effects of "environmental agents" may be compared.

The feasibility of the use of the isolated perfused lung in inhalation toxicology studies will be investigated.

Comparison of the responses to chemicals and drugs in the perfused lung *in vitro* will be made with those observed *in vivo* by Dr. Roth.

Serial No.: NIEHS-PT-030
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Binding of Basic Amines to Membrane Fractions from Rabbit Lung"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Pharmacokinetics Section, Pharmacology and Toxicology Branch

MAN YEARS:

Total:	0.75
Professional:	0.25
Other:	0.50

PROJECT DESCRIPTION

OBJECTIVES: Basic amines are removed from the blood by the lung with varying degrees of efficiency. This process can be demonstrated in the whole animal and in isolated-perfused lung. Little is known about the subcellular localization of these accumulated compounds and the nature of the binding site(s) have not been determined. This project was initiated in an attempt to deal with these problems. The immediate objectives are:

1. To isolate lung subcellular fractions which are kinetically similar to the isolated-perfused lung in their ability to bind basic amines.

2. To determine the cellular origin of active constituents of lung fractions which bind basic amines.

3. To determine the composition of specific binding sites.

METHODS EMPLOYED: Basic amine binding to lung fractions is being studied using differential centrifugation and equlibrium dialysis. Quantification of binding is accomplished with ^{14}C amines and liquid scintillation techniques. Lung fractions will be subdivided by digitonin binding and sucrose density centrifugation.

MAJOR FINDINGS: The 100,000 x g pellet obtained from lungs perfused with ^{14}C imipramine or amphetamine contains the largest amount of bound drug. In the

case of amphetamine the highest measure of radioactivity is located in the supernatant but this has been shown by sephadex column chromatography to be unbound. Equilibrium dialysis of the 600 x g, 7,000 x g and 100,000 x g lung particulate fractions from control animals demonstrates that the highest degree of affinity for imipramine also exists in the 100,000 x g pellet fraction. Analysis of results of equilibrium dialysis of ^{14}C imipramine with the lung 100,000 x g pellet fraction from control rabbits has demonstrated the existence of two saturable binding processes and one unsaturable uptake process. This finding is the same as that for the isolated perfused lung. The Ks values calculated for the saturable processes are essentially identical whether the 100,000 x g pellet fraction or the isolated perfused lung are used to study imipramine binding. In addition, it has been shown in both of these systems that desimipramine acts as a competitive inhibitor to imipramine uptake.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The binding of basic amines by the lung is important with respect to chemical and drug disposition and interaction in the lung. In order to fully understand the significance of this process it is necessary to determine the specific locations of chemical and drug binding, the mechanisms of transport, and the nature of any binding that may take place in the lung.

PROPOSED COURSE: An attempt is now underway to show that the similarity between binding to the isolated perfused lung and the 100,000 x g pellet fraction of the lung extends to drugs and chemicals other than imipramine and amphetamine. Because it is not clear which portion of the 100,000 g pellet fraction is responsible for chemical and drug binding, this fraction will be further subdivided. Plasma membrane and endoplasmic reticulum will be examined. Binding will be calculated on the basis of specific markers for these two membrane fractions. In addition, radioactive profiles of chemical or drug bound to fractions that have been treated with digitonin will be obtained. This should provide evidence pertaining to the binding of drugs or chemicals to membranes containing large concentrations of cholesterol (plasma) vs. membranes with low cholesterol content (E.R.).

Serial No.: NIEHS-PT-031
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Species Differences in Stimulation of Toxication-Detoxication Systems by Environmental Components. Extrahepatic Systems for Metabolism of Xenobiotics, Especially Intestine"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. S. Chhabra, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., R. J. Pohl, B.S., and J. Van Anda

COOPERATING UNITS: Biometry Branch

MAN YEARS:

Total:	2.0
Professional:	1.1
Other:	0.9

PROJECT DESCRIPTION

OBJECTIVES: The main objectives of this research are to study the effects of various factors such as physiological (age, sex, nutrition, hormones), genetic (species, strain) and environmental (insecticides, carcinogenic polycyclic hydrocarbons) on the enzymes metabolizing xenobiotics in various tissues. The current projects under study concern (1) the factors responsible for variations seen in induction of hepatic xenobiotic metabolizing enzymes by DDT, benzpyrene, 3-MC and phenobarbital in rats and mice, (2) possible sex differences in xenobiotic metabolism by extrahepatic tissues in the rat (lung and gut), and (3) species variation in xenobiotic metabolizing enzymes in liver vs. gut. This project was undertaken to find out the suitable species of animal for study in detail of xenobiotic metabolism by gut.

METHODS EMPLOYED: Livers, lungs, or guts are isolated from the control or treated animals (treated with DDT, benzpyrene, 3-MC or phenobarbital for varying periods of time) or from animals of different sex or species. The tissues are then homogenized. Either whole tissue homogenates are used for estimation of xenobiotic metabolizing enzymes or these homogenates are subjected to differential centrifugation to obtain subcellular particulate fractions. Various substrates are routinely used to quantify different kinds of xenobiotic metabolism. The amount of metabolites recovered after a definite time of incubation or amount of substrate disappearing during that time is taken as the amount of enzymic activity present in tissues, and is expressed either per

mg of microsomal protein or per gm of tissue (when whole homogenates are used).

Spectrophotometric methods are used for estimation of cytochrome P450 and cytochrome c reductase as well as in most of the enzyme activity measurements.

MAJOR FINDINGS:

1. Species differences in induction of hepatic xenobiotic metabolizing enzymes in mice and rats by DDT, benzpyrene and phenobarbital are as follows: Most laboratory animals respond to treatment with DDT or benzpyrene by increased levels of hepatic microsomal drug metabolizing enzymes. The variations in response to DDT noticed in previous studies in different animal species were quantitative except in Swiss Webster mice which did not seem to respond to either DDT or benzpyrene treatment. In our present study we found that the relative resistance shown by mice to hepatic enzyme induction could be overcome by high dose levels of DDT or benzpyrene (100 mg/kg and higher) and especially by treatments of longer duration. In rats, the maximal effect of both enzyme inducers was relatively long lived after termination of the treatment with inducers (several days with benzpyrene - and usually more than a week with DDT). In mice, the "enzyme induction" was lost quickly; enzyme levels returned to normal somewhere between 24-48 hours after the last treatment with either inducer. In contrast to the rat, benzpyrene treatment in mice did not shift the liver microsomal cytochrome P450-CO absorption spectral peak from 450 nm to 448 nm. The pH optima for hepatic microsomal aniline hydroxylase was not shifted by benzpyrene treatment of mice in contrast to the shift seen after such treatment in rats.

Further studies on hormonal regulation of xenobiotic metabolizing enzymes in mice showed that adrenalectomy, castration or both adrenalectomy and castration did not lower the basal hepatic xenobiotic metabolizing enzyme levels (while in the rat with similar operations, basal levels are lowered by 50-90% depending on the enzyme). Similarly, the magnitude of stimulation of xenobiotic metabolism by DDT, benzpyrene, 3-MC or phenobarbital in mice without adrenals, testes or both was not lowered compared to sham-operated mice, but instead was somewhat higher. These findings suggest that there is a marked species variation in hormonal regulation of hepatic xenobiotic metabolism in rats and mice.

2. Possible sex differences in xenobiotic metabolism by extrahepatic tissues in the rat (lung and gut): Some of the chemical and drug metabolizing enzymes have 2-3 times higher activity in livers from male than in female rats. We did not discover any such sex difference in the activities of enzymes in lung or in gut. Thus, sex differences in xenobiotic metabolism seem to be peculiar to the liver in the rat.

3. Species variations in xenobiotic metabolizing enzymes in liver vs. gut: We used rats, mice, hamsters, guinea pigs and rabbits in a preliminary study to select the model animal for more detailed study of xenobiotic metabolizing enzymes in gut. The xenobiotic oxidation reactions studied included hydroxylation of aniline, biphenyl and benzpyrene and N-demethylation of ethyl

morphine. Cytochrome c reductase and cytochrome P450 were measured as parts of the microsomal electron transport system. The xenobiotic metabolizing enzymes studied were present in livers of all the species and showed mainly quantitative differences. But in intestines from mice, rats, guinea pigs and hamsters either some of the enzymes were absent or had very low activity which would require very sensitive methods for detection. Rabbit emerged as the best animal model to study the xenobiotic metabolism in gut because all xenobiotic metabolizing enzymes studied were present in easily measurable quantities.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Species differences in response of xenobiotic metabolizing systems to pollutants (like DDT and benzpyrene) may explain both species specific toxicities of pollutants and interactions of pollutants with drugs and chemicals that differ so widely among species.

Xenobiotic metabolism by organs in continuous contact with environmental contaminants may explain organ specific damage by such pollutants. The gut is one such organ about which such knowledge is very slight, yet greatly needed. Pollutants in food and water as well as air contact gut and gut enzymes as a first line of defense of the body against such materials.

PROPOSED COURSE: Studies on species differences in response to stimulators or inhibitors of xenobiotic metabolism and in the hormonal regulation of this metabolism in the livers of mice vs. rats will continue. Age-related changes in the response of xenobiotic metabolizing enzymes to effects of DDT, benzpyrene and 3-MC will also be studied in liver (and gut where possible).

The studies on xenobiotic metabolism in rabbit gut will be a major part of our research efforts. The gut xenobiotic metabolizing enzymes will be characterized by studying the optimal conditions for *in vitro* assays - e.g. - temperature, pH, cofactor and substrate concentrations. This will be followed by study of the effect of treating rabbits with various stimulators and inhibitors. Changes in enzymes with age, deficient diets, and stress on different xenobiotic metabolizing enzymes in the rabbit gut will be studied.

PUBLICATIONS

Chhabra, R. S., and Fouts, J. R.: Stimulation of hepatic microsomal drug metabolizing enzymes in mice by 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) and 3,4-benzpyrene. *Tox. Appl. Pharmacol.* 25: 1973 (in press).

Chhabra, R. S., and Fouts, J. R.: Induction of hepatic microsomal drug metabolizing enzymes (HMDME) in a strain of mouse previously believed to be resistant to DDT or 3,4-benzpyrene (BP) treatment. Fifth International Congress on Pharmacology, Abstracts of Volunteer Papers, p. 41, 1972.

Serial No.: NIEHS-PT-032
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of these Systems"

PREVIOUS SERIAL NUMBER: NIEHS-PT-001 and NIEHS-PT-002

PRINCIPAL INVESTIGATORS: J. R. Fouts, Ph.D., T. Devereux, M.S., and R. J. Pohl, B.S.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Biometry Branch, Pathologic Physiology Branch

MAN YEARS:

Total:	1.9
Professional:	0.3
Other:	1.6

PROJECT DESCRIPTION

OBJECTIVES: This is a general effort directed to classifying and understanding the actions of chemicals and various physiological factors that affect the body mechanisms for metabolizing "xenobiotics" and other substances (endogenous and exogenous) which can be substrates for systems often called "drug metabolizing enzymes." Such metabolizing enzyme systems are found relatively concentrated in excretory organs and tissues found at the interface between man and his environment (e.g., skin, gut, lung, kidney, and liver). These enzymes respond to a variety of chemicals (such as pollutants, pesticides, food additives) and changes in physiology (age, sex, nutrition) by increases or decreases in their activity which can affect the action and toxicity of xenobiotics that reach these enzymes. Combinations of chemicals can affect these xenobiotic metabolizing systems in ways both qualitatively and quantitatively different from effects of single entities. Present efforts are devoted to studies on development of these systems in the newborn - especially comparing toxifying and detoxifying systems in liver and lung. Metal ion effects on the systems in both tissues are also under investigation - including metal effects on overall xenobiotic metabolism as well as metal ion effects on defined components of one of the major metabolic schemes - namely the mixed-function oxidase (MFO), cytochrome P450-dependent system.

METHODS EMPLOYED: Most work has been done on liver and lung microsomal preparations though this was preceded in the case of lung with studies on other parts of the homogenate. Microsomes are isolated from tissues of animals of different ages (rats and rabbits) and used to study metabolism of different xenobiotics and drugs or the amount or activity of key steps in the MFO-P450 dependent system. Selected toxifying systems have been partially purified from lung and liver by classical technics of enzyme purification - sonication, solubilization with detergents, concentration of selective precipitation with $(NH_4)_2$ SO_4, etc. Spectrophotometric methods are used to study cytochromes (b_5, P450 and their oxidation and reduction), and quantify metabolites of substrates added to microsomes or purified enzyme systems.

MAJOR FINDINGS:

1. Age dependent changes in toxifying-detoxifying systems in liver and lung - with Ms. Devereux: This extends a study now in its second year and has concentrated on the metabolism of a model substrate by two mechanisms - N-demethylation and N-oxidation. N-demethylations are often detoxifying reactions whereas N-oxidations are usually toxifying - creating a much more reactive molecule than the parent compound. In rabbits, both liver and lung have appreciable levels of both N-demethylases and N-oxidases. N-demethylase activity (per mg protein) is the same in liver and lung for many chemicals and drugs (e.g. - benzphetamine and dimethylaniline demethylations) while N-oxidase activity in rabbit lung is much greater than liver especially at "non-physiologic" pH (between pH 8 and 9). The response of liver and lung demethylases to metals like Ni^{+2}, Mg^{+2} and Hg^{+2} is similar but the N-oxidases of liver and lung are quite different. Lung N-oxidase can be stimulated by low concentrations of Hg^{+2} and Ni^{+2} and high concentrations of Mg^{+2} whereas the liver enzyme is unaffected or inhibited by most metals. The effects of Hg^{+2} have been studied in some detail to show that it is probably not stimulating N-oxidase via an SH-reagent action and that the difference between lung and liver N-oxidase enzymes disappears as the enzyme is purified.

In the newborn rabbit both liver N-oxidase and N-demethylase are at very low levels and increase slowly for the first 2 weeks of life. A sudden jump to adult levels occurs between 3 and 4 weeks of age in rabbits. In lung, these systems start out quite differently - N-oxidase is at 50 percent of adult levels almost at birth and rises to adult levels at a steady rate while lung N-demethylase in newborn rabbits is at low levels and rises slowly to adult levels - only 60 percent of adult level is present even at 4 weeks of age.

N-demethylases in both lung and liver are sensitive to mercurials, but not especially to -SH reagents (N-ethylmaleimide and iodoacetamide). The N-oxidase of liver can be inhibited by mercury, but much less easily than the N-demethylase; liver N-oxidase is not sensitive to -SH reagents like N-ethylmaleimide or iodoacetamide. As already mentioned, neither Hg nor -SH reagents are effective inhibitors of lung N-oxidase.

2. TPNH-cytochrome P450 reductase as a rate-limiting step in xenobiotic metabolism in liver and lung microsomal MFO-P450-dependent systems - with Ms. Pohl: We have continued our studies of how metal ions and substrates interact at this "rate-limiting" step in xenobiotic metabolism. A number of

conditions appear to be able to block metal ion effects on P450 reductase including high ionic strength, 3-methylcholanthrene or benzpyrene pretreatment of animals, and high osmotic pressures. Many divalent cations can stimulate P450 reductase in the 1-50 mM concentration range. Monovalent cations do not usually affect the system until concentrations greater than 50 mM are reached at which point some small stimulation occurs. One major interaction effect of monovalent cations is their ability to block stimulation by divalent cations also added to the system. This blocking effect on divalent cation effects on P450 reductase is also seen when osmotic pressure is increased by raising buffer concentration (Hepes buffer is not a metal binding buffer) or adding sucrose solutions. Some organic solvents like acetone may also block metal ion effects on P450 reductase. Our studies suggest that metal ions may be affecting the structure of the microsome in such a way as to cause the enhanced P450 reductase activity. A key stage of this research depends on Dr. Philpot's ability to purify lung and liver P450 and P448 for our use.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolism of xenobiotics by enzymes in tissues can affect the toxicity and action of these chemicals both within the metabolizing tissue and elsewhere. Effects of environmental pollutants and chemicals on these systems can alter "normal" responses of an organism (to its own hormones, drugs it must or does ingest and chemicals and pollutants it must contact) if these enzyme systems are stimulated or inhibited to produce more toxic molecules or to get rid of these more slowly than normal. Such interactions have been documented as the basis for several synergisms between pairs of chemicals, chemicals and drugs, pollutants and normal body hormones, etc. This program seeks to understand some of the factors that affect xenobiotic metabolism in tissues in contact with our environment. The program so far is comparing lung and liver systems to see where differences exist and what these might mean for toxic actions and interactions of inhaled vs. ingested materials. The fact that a toxifying system in lung (N-oxidation) exists in high activity whereas most detoxifying systems in this organ are at low levels; that this difference though present in adults is exaggerated in the newborn; and that metals like Hg^{+2} and Ni^{+2} turn the N-oxidase in lung "on" even more while depressing several of the detoxications in both liver and lung would seem to be most important. Studies of the rate-limiting step in MFO-P450-dependent systems which suggest that some effectors of xenobiotic metabolism are acting via changing microsomal membrane configuration rather than directly is one more step along the path of understanding the varied ways that chemicals can change the functions of these most critical systems.

PROPOSED COURSE: Further purification of lung and liver toxication (N-oxidase) systems to identify the component in lungs (lipid?) which accounts for Hg^{+2}, Ni^{+2} and even Mg^{+2} stimulations of the system. Purification of comparison of lung and liver systems from control and 3-MC treated animals to understand how induction prevents metal ion effects on the rate-limiting step (to be done with help from Dr. Philpot). Further studies of membrane alterations using metal ion chelators and microsomes from metal deficient animals to further assess the idea that metals may act allosterically on xenobiotic metabolizing systems. Effects of inducers (phenothiazines, pregnenolone carbonitrile, and β-naphthoflavone) and inhibitors (CCl_4) on lung vs. liver N-oxidase and N-demethylases. Role of metals in uncoupling toxication (N-oxidation) from detoxication (including conjugation) systems.

PUBLICATIONS

Fouts, J. R., and Devereux, T. R.: Developmental aspects of hepatic and extrahepatic drug metabolizing enzyme systems: Microsomal enzymes and components in rabbit liver and lung during the first month of life. J. Pharmacol. Exptl. Therap. 183: 458-468, 1972.

Fouts, J. R., and Devereux, T. R.: Use of 10-second sonication of homogenates to increase microsomal protein yield in liver and lung from young and adult Dutch Belt rabbits. Biochem. Pharmacol. (in press).

Fouts, J. R.: Some selected studies on hepatic microsomal drug-metabolizing enzymes - environmental interactions. Drug Metabolism and Disposition (in press).

Fouts, J. R.: Microsomal mixed-function oxidases in the fetal and newborn rabbit. Lars O. Boreus (Ed.): In Fetal Pharmacology, Raven Press, N.Y., 1973, pp. 305-321.

Serial No.: NIEHS-PT-033
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Interaction of Water-Soluble Substrates with Water-Insoluble Enzymes in Aqueous Media"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: G. E. R. Hook, Ph.D., J. R. Bend, Ph.D., and J. R. Fouts, Ph.D.

OTHER INVESTIGATORS: R. E. Easterling, L. B. Gilmore, B.A., and W. D. Taylor

COOPERATING UNITS: None

MAN YEARS:

Total:	1.2
Professional:	0.5
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: Most foreign compounds which are acted on by the microsomal mixed-function oxidase system are soluble to only a very small extent in water at physiological pH. The interactions of water-insoluble substrates with these enzymes have been studied extensively *in vitro*, but generally only with the aid of solubilizing or suspending agents designed to bring the substrate into aqueous solution. Often the effects of the solubilizing agent on the enzyme system under study have been almost completely ignored. The initial objectives of this study were to (1) investigate the effects of a number of currently popular solubilizing and suspending agents on liver microsomal electron transport as related to the hydroxylation of biphenyl, (2) if possible find a solubilizing or suspending agent which was itself a non-effector of microsomal electron transport, and (3) investigate the interaction of water-insoluble substrates with microsomes structurally modified by such membrane modifiers as detergents.

METHODS EMPLOYED: Microsomes were isolated from rabbit livers. Various aspects of microsomal electron transport were investigated. NADPH-cytochrome P450 reductase, NADPH-cytochrome c reductase, biphenyl 4-hydroxylase and cytochrome P450 were studied by classical methods using spectrophotometry and spectrophotofluorometry.

MAJOR FINDINGS: The organic solvents ethanol and dimethylsulfoxide inhibited biphenyl 4-hydroxylase and NADPH-cytochrome P450 reductase. Acetone inhibited

the hydroxylase uncompetitively at concentrations which appeared to stimulate NADPH-cytochrome P450 reductase. The non-ionic detergents Tween 20, Tween 80 and Triton X-100 inhibited biphenyl 4-hydroxylase although only Triton X-100 markedly affected the reduction of cytochrome P450 by NADPH. The interaction of Tween 80 with the hydroxylase gave rise to nonlinear Lineweaver-Burk plots although at high concentrations of biphenyl or low concentrations of the detergent the inhibition appeared to be competitive. Biphenyl caused a 2-3 fold stimulation of NADPH-cytochrome P450 reductase, but in the presence of Tween 80 the stimulation was absent. Because Vmax of biphenyl 4-hydroxylase in the presence of Tween 80 was not significantly different from Vmax in its absence, it would appear that the reduction of cytochrome P450 was not rate-limiting.

Of all the carriers studied, only carboxymethyl cellulose (CMC) was without effect on all aspects of microsomal electron transport investigated. As far as biphenyl 4-hydroxylase is concerned, CMC appears to be the most suitable substrate carrier. These findings are a summary of the results described in the manuscript submitted to Chemico-Biological Interactions.

Detergent interactions with liver microsomes have been investigated considerably further. The ability of the non-ionic detergents Tween 20, Tween 80 and Triton X-100 to solubilize microsomal membranes and convert cytochrome P450 into P420 differs considerably. The P450 to P420 conversion rates in the presence of these detergents was greatly affected by the presence of biphenyl; with Triton X-100 the rate was increased and Tween 80 the rate was decreased but with Tween 20 no effect was seen.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Detergents are tools which may be used for the selective dismantling of biological membranes. Data so far indicates that the non-ionic detergents studied differ considerably in their interactions with microsomal membranes and the chemical or drug-metabolizing enzyme system. With judicious use, the specific interactions of detergents with the microsomal membrane may provide considerable information concerning mechanisms of stimulation and inhibition of, and the nature of the chemical- and drug-metabolizing enzyme system as it exists in the microsomal membranes.

Knowledge concerning the nature of stimulation and inhibition of the chemical- or drug-metabolizing enzyme system is valuable since the interaction of man with his environment is to a large extent regulated by his ability to overcome its toxic components by processes such as metabolism of these components.

PROPOSED COURSE: Detergent interactions with microsomal components will be pursued. Studies of biphenyl metabolism and factors affecting it will continue both in terms of a general model system and as a prototype of the class of pollutants, PCB's. A paper entitled "The Effect of Some Biphenyl Solubilizing and Suspending Agents on Biphenyl-4-Hydroglase of Rabbit Liver Microsomes" has been submitted for publication to Chemico-Biological Interactions.

PUBLICATIONS

Hook, G. E. R., Bend, J. R., Gram, T. E., and Fouts, J. R.: The effect of some biphenyl solubilizing and suspending agents on the activity of biphenyl-4-hydroglase of rabbit liver. Fifth International Congress on Pharmacology. Abstracts of Volunteer Papers, p. 105 (1972).

Serial No.: NIEHS-PT-034
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Study of Drug Metabolism in Liver or Hepatopancreas and Gill of Some Marine Species"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. R. Bend, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., and R. J. Pohl, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.20
Professional:	0.10
Other:	0.10

PROJECT DESCRIPTION

OBJECTIVES: To compare microsomal drug metabolizing activity in several vertebrate and invertebrate marine species and to characterize the hepatic mixed-function oxidase system of the little skate (Raja erinacea) in some detail because relatively large amounts of enzyme activity were found in the liver of this species.

METHODS EMPLOYED: Subcellular fractions (either 10,000 g supernatant or microsomes) are prepared from gill, liver or hepatopancreas of freshly collected marine specimens. Aniline hydroxylase and benzphetamine demethylase activities are measured by standard procedures.

MAJOR FINDINGS: Of the marine species examined, highest specific activities for aniline and benzphetamine metabolism were found in liver microsomes from the little skate although winter flounder (Pseudopleuronectes americanus) and dogfish shark (Squalus acanthias) hepatic microsomes also contained activity (7 - 20 percent of skate liver microsomal specific activity). Negligible aniline hydroxylase and benzphetamine demethylase activities were associated with hepatopancreas of the rock crab (Cancer borealis) or the lobster (Homarus americanus) or in the gill of all species studied.

Interesting properties of the skate liver microsomal system (towards the substrates, aniline and benzphetamine) were that the activities remained linear up to microsomal protein concentrations of at least 10 mg/ml incubation mixture, and with time for at least 45 minutes with aniline and 30 minutes with

benzphetamine (at 30° C). Highest specific activities were noted at incubation temperatures of 30-37° C, although the summer ocean temperature at the collection site is near 15° C. Cofactor concentration vs. activity curves, pH vs. activity curves, substrate concentration vs. activity curves and inhibitor (cytochrome c) concentration vs. activity curves have also been obtained with aniline and benzphetamine as substrates in skate liver microsomal incubation mixtures.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our country's waterways are often contaminated with pesticides, industrial chemicals and other foreign chemicals which have been synthesized in large quantities over the last few decades. The ability of marine species, particularly those that are used as human food sources, to metabolize and excrete xenobiotics is therefore relevant not only to the fate of these species but to food residues for humans.

PROPOSED COURSE: Cytochrome P450 levels will be quantitated in skate, flounder and dogfish shark liver microsomes. Hepatopancreas microsomes from lobster and crab will also be examined qualitatively for the presence of this cytochrome. Because the sensitivity of the assays used for measuring aniline hydroxylase and benzphetamine demethylase is too low to tell conclusively if there is any drug metabolizing activity in crab and lobster hepatopancreas or in gill, we intend to study the dealkylation of ethoxyumbelliferone in these cases because the sensitivity of this fluorometric assay is at least 100 times greater than that for aniline or benzphetamine. The effect of administering (orally, parenterally and in the sea-water) to marine species known environmental contaminants on liver microsomal metabolism of chemical and drugs in the skate and flounder will also be studied.

PUBLICATIONS

Bend, J. R., Pohl, R. J., and Fouts, J. R.: Some Properties of the microsomal drug-metabolizing enzyme system in the little skate. Bull. Mount Desert Island Bio. Lab. (in press).

Serial No.: NIEHS-PT-035
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Uptake, Storage and Excretion of ^{14}C-2,4,5,2',5'-Pentachlorobiphenyl (^{14}C-PCB) in the Lobster (Homarus americanus) and in the Winter Flounder (Pseudopleuronectes americanus)"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., and J. R. Fouts, Ph.D.

OTHER INVESTIGATORS: A. M. Guarino, Ph.D., D. P. Rall, M.D., Ph.D., and R. J. Pohl, B.S.

COOPERATING UNITS: Laboratory of Toxicology, National Cancer Institute

MAN YEARS:

Total:	0.8
Professional:	0.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this investigation are to study the uptake, distribution and excretion of a single, radio-labeled pentachlorobiphenyl isomer in two edible marine species.

METHODS EMPLOYED: At various times after ^{14}C-PCB administration, specimens are sacrificed, tissues are dissected out and weighed aliquots are solubilized in NCS (Amersham-Searle) prior to determination of the radioactivity present by liquid scintillation counting. The presence or absence of metabolites is also determined by conventional chromatographic techniques.

MAJOR FINDINGS: After a single injection of ^{14}C-PCB (0.20 mg/kg) into the pericardial sinus of the lobster, the specific activity of the hepatopancreas (dpm/g) was much greater (at least ten times) than in any other tissue sampled. Moreover, there was no decrease in the C^{14}-content of the hepatopancreas over a one week time period as compared to lobsters that were sacrificed 24 hours after treatment. Similar results were found with intestine, stomach, gill and egg masses. However, there were significant decreases in the C^{14}-content of plasma, green gland, heart, tail muscle and claw muscle over the one week period after treatment.

Initial studies in flounder, where the ^{14}C-PCB (1.0 mg/kg) was administered orally in corn oil, demonstrated that absorption from the gastrointestinal

tract was erratic and seemed to be very low. Most of the administered activity that was recovered was found in the intestinal contents and feces, even only a few hours after treatment.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polychlorinated biphenyls (PCB) are known to exist as tissue residues in many marine species, including the lobster. The storage sites of PCB in edible marine species, as well as the ability of these animals to metabolize and/or excrete these compounds may well play an important role in determining whether or not these species can continue to be used as safe food sources in the future.

PROPOSED COURSE: The radioactive content of various tissues will be determined in lobsters at 2 weeks and 4 weeks after a single injection into the pericardial sinus to see if significant decreases do occur and to determine the biological (excretion) half-life. Similar experiments are planned in the winter flounder following intravenous injection of ^{14}C-PCB. Further studies will involve the uptake and distribution of radioactivity from sea water containing ^{14}C-PCB in these species.

PUBLICATIONS

Bend, J. R., Guarino, A. M., Briley, P.D., Rall, D. P., and Fouts, J. R.: Distribution of ^{14}C-2,4,5,2',5'-Pentachlorobiphenyl in the Lobster, Homarus americanus, after a Single Pericardial Injection. Bull. Mount Desert Island Biol. Lab. 1972 (in press).

Serial No.: NIEHS-PT-036
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of Heavy Metals on Microsomal and Mitochondrial Enzymes: Protection Against Biochemical Lesions by Phenobarbital"

PREVIOUS SERIAL NUMBER: NIEHS-PP-018

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: B. W. Fowler, Ph.D., S. W. Hwang, Ph.D., R. Klein, Ph.D., and O. S. McDaniel

COOPERATING UNIT: Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina

MAN YEARS:

Total:	0.8
Professional:	0.6
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: The health hazards of organic mercury in our environment are becoming increasingly evident. However, most attention has been focused on neurological effects, with essentially no studies conducted on in vivo effects on the hepatic drug metabolizing enzymes. Investigations during the past year attempt to: (1) determine the mechanism by which organic mercurials decrease cytochrome P450 and cytochrome b_5 content, (2) determine effects on the drug-metabolizing enzymes resulting from the interaction of inducing agents (chlordane and phenobarbital) and organic mercurials, (3) determine possible differences in the effects on microsomal enzymes by changing the route of administration or dose level of MMH, and (4) evaluate phenobarbital protection against MMH-induced nephrotoxicity and neurotoxicity.

METHODS EMPLOYED: Liver and kidneys from male and female rats, mice, and guinea pigs were used for preparation of microsomes and mitochondria. MMH was administered either subcutaneously or in the drinking water (5 or 10 ppm for 15 or 30 days). Phenobarbital was administered i.p. at 75 mg/kg once every 4 days. Oxidative phosphorylation rates were measured polarographically.

MAJOR FINDINGS: Previous studies described the effects of MMH on hepatic microsomes and microsomal subfractions. Essentially, we had demonstrated that

MMH decreased mixed-function oxidase activity by apparently decreasing cytochrome P450 content.

Increased degradation of CO-binding particles by MMH. Incorporation of H^3-δ-aminolevulinic acid (H^3-ALA) into hemoprotein of hepatic CO-binding particles (cytochrome P450) was more rapid in MMH-treated (10 mg/kg/day for 2 days, subcutaneously) than in control rats, although maximum incorporation was similar in both cases. Treatment with MMH stimulates the synthesis of fast-phase hemoprotein and decreases synthesis of the slow-phase component. The half-life of the fast-phase hemoprotein was 5.5 hours in treated animals compared to 10 hours in controls whereas the slow-phase half-lives were approximately 65 hours in both sets of animals. These data suggest that cytochrome P450 content is reduced by increasing degradation of the fast-phase component and/or decreasing synthesis of the slow-phase component. A control experiment was devised which demonstrated that the biphasic degradation curves were not influenced by heme exchange during preparation of subcellular particles from control, chlordane-treated, and MMH-treated rats.

Effects on rat liver microsomes and kidney mitochondria when MMH was administered in the drinking water. When rats were administered MMH in the drinking water at 5 ppm for 30 days (0.7 mg/kg per day; total dose of 20 mg/kg) or 10 ppm for 15 days (1.4 mg/kg/day; total dose of 20 mg/kg) decreases in mixed-function oxidase activity were small. Cytochrome P450 and aminopyrine demethylation were decreased approximately 20 percent in both male and female rats (significant at $P < 0.05$). Hepatic total mercury levels were approximately 50 μg/g tissue following subcutaneous administration (10 mg/kg/day for 2 days; total dose of 20 mg/kg) and approximately 20 μg/g tissue when rats received the equivalent dose in the drinking water over 30 days. After subcutaneous injection 40 percent of total hepatic mercury was in the inorganic form whereas the percentage was 10 percent after administration in the drinking water.

Phenobarbital treatment to rats protected against MMH-induced (5 ppm in the drinking water for 30 days) kidney mitochondrial damage in the region of the proximal tubules as determined by ultrastructural studies. However, methylmercury at this dose schedule caused no significant changes in oxidative phosphorylation rates (state 3, state 4, ADP:O) in kidney mitochondrial preparations. Phenobarbital also appeared to protect against MMH-induced neuropathology. Brain levels of both methylmercury and inorganic mercury were significantly decreased when rats received phenobarbital injections during the course of MMH exposure. Liver and kidney mercury levels were not changed by phenobarbital treatment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: MMH exposures interfere with oxidative enzymes that function in the degradation of xenobiotics as well as the normal metabolism of many endogenous compounds. These alterations in mixed function oxidase activity may interfere with the normal regulation of the steroid hormones. The carbamate pesticides and many other environmental agents and drugs are considered relatively safe because they are rapidly detoxified by hydroxylation reactions. However, if the normal rate of hydroxylation is inhibited, these compounds may become considerably more toxic to animals. Decreased mixed-function oxidase activity caused by MMH may also have implications in the administration of drugs to patients which

have been exposed to methylmercury because many of the drugs are metabolized by the mixed-function oxidases. In our experiments hexobarbital sleeping times (rats) were increased by 250 percent in animals treated with MMH at dosage levels which caused no neurological symptoms. In more recent studies rats were administered MMH in the drinking water at low-level chronic exposures to better simulate environmental conditions. Phenobarbital protection against nephrotoxicity and neurotoxicity may have clinical applications especially if phenobarbital can reverse some of the MMH-induced pathology.

PROPOSED COURSE: The protective effect of phenobarbital against MMH-induced pathologies could be explained by several possibilities. The two most likely are described below:

1. Phenobarbital either induces metallothionein synthesis in a way which increases the amount of protein available to bind methylmercury or it may induce synthesis of a different form of metallothionein that has increased binding affinity. We plan to investigate this possibility by purifying metallothionein (Sephadex and isoelectric focusing) and studying rates of synthesis and degradation in liver, kidney, and brain using labelled cysteine.

2. Phenobarbital might increase demethylation of MMH to form inorganic mercury which does not cross the blood-brain barrier as readily as methylmercury. This possibility is being investigated by following in vitro demethylation rates in liver, kidney, and brain from phenobarbital-treated and control rats.

PUBLICATIONS

Lucier, G., Klein, R., McDaniel, O. S., and Brubaker, P.: Effects of methylmercury hydroxide on rat liver microsomal enzymes. Chem.-Biol. Interact. 4: 265-280, 1972.

Lucier, G. W., and McDaniel, O. S.: Decreased hepatic mixed-function oxidase activity by methylmercury in mammals. Fifth International Congress on Pharmacology, July 1972.

Lucier, G. W., Klein, R., Matthews, H. B., and McDaniel, O. S.: Increased degradation of rat liver CO-binding particles by methylmercury. Life Sciences 11: (Part II) 597-604, 1972.

Lucier, G. W., McDaniel, O. S., Williams, C., and Klein, R.: Effects of methylmercury and chlordane on the metabolism of carbaryl and carbofuran in rats. Pest. Biochem. Physiol. 2: 244-255, 1972.

Lucier, G. W., McDaniel, O. S., Matthews, H. B., and Klein, R.: Effects of methylmercury on microsomal mixed-function oxidase components in rodents. Molec. Pharmacol. (in press).

Fowler, B. A., Lucier, G. W., Brown, H. W., and Mushak, P.: Phenobarbital protection against methylmercury nephrotoxicity: An ultrastructural study. Federation Procs. Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 1973.

Serial No.: NIEHS-PT-037
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Tetrachloro-Dibenzo-p-dioxin (TCDD) Induced Alterations in Microsomal Enzymes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: B. W. Fowler, Ph.D., G. E. R. Hook, Ph.D., J. A. Moore, D.V.M., B. R. Sonawane, Ph.D., and O. S. McDaniel

COOPERATING UNITS: NONE

MAN YEARS:

Total:	0.6
Professional:	0.5
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES:

1. Characterize changes in mixed-function oxidase and glucuronyltransferase activities in hepatic and extrahepatic tissues following treatment with TCDD.

2. Determine time-course of effects and dose response relationships.

3. Determine possible sex differences in microsomal responses to TCDD.

4. Correlate changes in enzyme activity with ultrastructural alterations.

5. Determine effects of TCDD on microsomal subfractions.

METHODS EMPLOYED: Male and feamle rats were used in these studies. 2,3,9,8-tetrachloro-dibenzo-p-dioxin (TCDD) was administered orally at 0.2, 1.0, 5.0, or 25 μg/kg and rats were sacrificed at designated times after this single dose. Microsomal enzymes and cytochromes were assayed by standard techniques.

MAJOR FINDINGS: A single oral dose of TCDD at 25 μg/kg caused striking induction of mixed-function oxidase components and glucuronyltransferase. The following data are from male liver microsomal enzymes 3 days after treatment:

Enzyme or component	Percent change (per mg protein)
Cytochrome P450	+80
Cytochrome b_5	+80
Aminopyrine demethylation	-40
Benzphetamine demethylation	-40
Ethylmorphine demethylation	-30
Benzpyrene hydroxylation	+600
Aniline hydroxylation	+100
Biphenyl-2-hydroxylation	+1000
Biphenyl-4-hydroxylation	+90
TPNH cytochrome c reductase	No change
Glucuronyltransferase (+ Triton, $+Mg^{++}$)	+800
Glucuronyltransferase (+ Triton, $-Mg^{++}$)	+600
Glucuronyltransferase (- Triton, $+Mg^{++}$)	+800
Glucuronyltransferase (- Triton, $-Mg^{++}$)	+700
Km p-nitrophenol	No change
Km UDPGA	No change
Phospholipid	No change
Cholesterol	No change
β-glucuronidase	No change
Microsomal protein	+15%
SER protein	No change
RER protein	+30%

Induction of enzyme activity was evident in both smooth- and rough-surfaced hepatic endoplasmic reticulum; kidney glucuronyltransferase activity was increased by 300 percent. An inductive effect was still observed 28 days after a single oral treatment of 5 or 25 μg/kg, and time-course data were similar for 5 and 25 μg/kg. Female liver benzpyrene hydroxylation (8-fold), biphenyl-2-hydroxylation (8-fold), and glucuronyltransferase (3-fold) were significantly induced by a single oral dose of 0.2 μg/kg whereas male liver enzymes were not affected by this dose. Electron micrographs of livers from treated rats revealed proliferation of rough endoplasmic reticulum. Biochemical fractionation verified this observation. Oxidative phosphorylation rates in isolated liver mitochondria were not changed by TCDD treatment at any of the specified doses or during the time-course experiment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polychlorinated dibenzodioxins and dibenzofurans are contaminants of many organochlorine chemicals such as 2,4,5-T and the PCB's. TCDD is one of the most toxic dioxins having an oral LD50 in rats approximately 100 mg/kg and has been reported to be teratogenic at low doses. Our results demonstrate that TCDD is perhaps the most potent inducing agent of hepatic microsomal enzymes yet reported; potent induction is evident in female rats after a single oral dose of 0.2 μg/kg. Because significant levels of induction were observable 28 days after a single dose, chronic exposures to extremely small amounts of dioxins

could therefore present environmental health hazards. Marked increases in hydroxylation and glucuronidation rates could disrupt normal steroid regulation by increasing steroid excretion. Subsequent hormonal imbalances could be dangerous to developing fetuses and might be related to teratogenic properties of TCDD.

PROPOSED COURSE: Investigate possible induction of fetal rat liver microsomal enzymes following low-level maternal exposures to TCDD. We also plan to investigate possible induction of placental glucuronyltransferase.

PUBLICATIONS

Lucier, G. W. McDaniel, O. S., Hook, G. E. R., Sonawane, B. R., and Fowler, B. W.: TCDD-induced changes in microsomal enzymes. Environ. Health Perspect., Issue No. 5, August 1973.

Serial No.: NIEHS-PT-038
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Reprot
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of Hycanthone and Two of Its Chlorinated Analogs on Hepatic Microsomes in Rats"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: J. R. Bend, Ph.D., and O. S. McDaniel

COOPERATING UNIT: None

MAN YEARS:

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: This study was initiated to determine if possible hepatotoxic properties of hycanthone methanesulphonate are reflected by effects on hepatic microsomal enzymes in rats. Another objectives was to compare the microsomal effects of hycanthone with two chlorinated analogs that are also effective antischistosomal agents.

METHODS EMPLOYED: Hycanthone, IA-3, or IA-4 were administered IM to male rats and activities of hepatic microsomal enzymes and cytochrome contents were monitored with respect to time after dosing (1 through 22 days) and dose (25, 50, and 100 mg/kg). Parameters investigated were cytochrome P450, cytochrome b_5, aminopyrine demethylation, glucuronyltransferase, and β-glucuronidase. Effects on detoxication enzymes in vivo were investigated by determining hexobarbital sleeping times in control and treated rats. In other experiments, microsomes were subfractionated and effects of hycanthone and analogs on enzyme activities in smooth- and rough-surfaced endoplasmic reticulum were determined.

MAJOR FINDINGS: Two days after treatment with 100 mg/kg hycanthone, aminopyrine demethylation was decreased by 48 percent and cytochrome P450 by 30 percent. Aminopyrine demethylation activity remained at approximately 50 percent of control levels through the seventh day after hycanthone injection. Enzyme activity returned to control levels by the fourteenth day. Microsomal protein was decreased by hycanthone treatment and this was attributable to decreased smooth endoplasmic reticulum protein. Effects of hycanthone on other microsomal

enzymes such as glucuronyltransferase, β-glucuronidase, and acid phosphatase were not as great as those observed on the mixed-function oxidase components. IA-4 (chlorinated hycanthone analog) did not significantly affect any of the microsomal parameters tested, whereas IA-3 (chlorinated lucanthone analog) decreased aminopyrine demethylation by 38 percent in smooth endoplasmic reticulum. Dose response studies demonstrated that aminopyrine demethylation and cytochrome P450 were significantly decreased by a single hycanthone injection at 50 or 100 mg/kg. A dose of 25 mg/kg caused only slight decreases in these parameters. Hexobarbital sleeping times were significantly longer than controls in rats receiving 50 or 100 mg/kg.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hycanthone methanesulphonate has been administered to millions of people in many countries as an antischistosomal agent. Recently this drug was shown to have mutagenic, teratogenic, and possibly hepatotoxic properties. Our research was designed to determine if possible hepatotoxicity of hycanthone and some of its analogs resulted in changes in activity of rat hepatic microsomal enzymes. The microsomal enzymes, of course, are extremely important in the metabolism of xenobiotics.

PROPOSED COURSE: This project is terminated because of competing priorities.

Serial No.: NIEHS-PT-039
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "A Comparative Study on Rat Liver Microsomal and Lysosomal β-Glucuronidase"

PREVIOUS SERIAL NUMBER:

PRINCIPAL INVESTIGATOR: G. W. Lucier, Ph.D.

OTHER INVESTIGATORS: O. S. McDaniel

COOPERATING UNITS: None

MAN YEARS:

Total:	0.6
Professional:	0.6
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study were (1) to purify β-glucuronidase from rat liver microsomes and lysosomes, (2) to compare physical properties of purified enzyme from both subcellular fractions (molecular weights, pH optima, temperature optima, heat stabilities, enzyme kinetics, sedimentation coefficients and electrophoretic mobilities, (3) to study rates of incorporation of labeled amino acids into enzyme from lysosomes and microsomes to evaluate rates of enzyme synthesis, and (4) to determine possible differences in electrophoretic properties of lysosomal and microsomal β-glucuronidase from guinea pigs, rabbits, rats and fetal and adult tissues.

METHODS EMPLOYED: Microsomal β-glucuronidase was solubilized by ribonuclease digestion and/or ultrasound and the lysosomal enzyme was solubilized by freeze-thawing and ultrasound. Purification was accomplished by ammonium sulphate fractionation, pH fractionation, migration on sucrose-density gradients, and isopycnic gradients. Electrophoretic mobilities at each step during purification were determined on 5 percent polyacrylamide gels using substrate stains.

MAJOR FINDINGS: Specific activities after purification increased approximately 150-fold above microsomal and lysosomal activities. Regardless of the solubilization method, purified lysosomal and microsomal β-glucuronidase had similar molecular weights (240,000), sedimentation coefficients (10.9), Km values for p-nitrophenyl-β-D-glucuronide (1.4×10^{-4}M), heat stabilities, temperature optima (65^{o} C), and pH optima (4.0). However the electrophoretic migration

rate at each purification step of lysosomal β-glucuronidase on 5 percent polyacrylamide gels was nearly twice that of the microsomal enzyme. These data suggest that there is a charge difference between purified lysosomal and microsomal β-glucuronidases, although molecular weights, sedimentation coefficients, Km values, and other properties are the same. Electrophoresis resolved only one protein for the lysosomal purification and three proteins for the microsomal preparation.

Attempts to label β-glucuronidase were unsuccessful following injection with ^{14}C-valine, ^{14}C-leucine, ^{14}C-amino acid mixture, or ^{14}C-glucosamine.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: β-Glucuronidase functions in the metabolic hydrolysis of compounds conjugated with glucuronic acid. It therefore acts as a deconjugation system which liberates a more lipophilic aglycone from polar conjugated compounds such as steroid glucuronides or glucuronides of some exogenous chemicals. Deconjugation of glucuronides of toxicants can often be considered a toxication reaction. In many disease conditions including cancer and diabetes, the levels of tissue and serum β-glucuronidase are elevated. It is therefore very important to characterize physical properties of the enzyme and determine substrate specificities in both lysosomes and microsomes.

PROPOSED COURSE: This project is temporarily suspended in favor of other projects.

PUBLICATIONS

Lucier, G. W., and McDaniel O. S.: Alterations in rat liver microsomal and lysosomal β-glucuronidase by compounds which induce hepatic drug-metabolizing enzymes. Biochim. Biophys. Acta, 261: 168-176, 1972.

Lucier, G. W.: A comparative study on microsomal and lysosomal β-glucuronidase. Federation Procs. Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 1973.

Serial No.: NIEHS-PT-040
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Perinatal Development of Biliary Excretion"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: S. W. Hwang, Ph.D., and R. L. Dixon, Ph.D.
(Pathologic Physiology Branch)

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS:

Total:	0.4
Professional:	0.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project was to examine various aspects of indocyanine green (ICG) excretion into the bile by guinea pigs ranging in age from 1 day old to adult. Specifically, the rate of bile flow, bile-to-plasma and liver-to-plasma ratios, plasma disappearance curves, hepatic uptake and transport maxima (Tm) were determined for animals of different ages.

METHODS EMPLOYED: Guinea pigs of various ages were anesthetized with pentobarbital. Through a midline abdominal incision, the common bile duct was cannulated with a blunt hypodermic needle shaft attached to PE tubing and the cystic duct was ligated. After i.v. injection of ICG, the bile was collected for several timed periods. Blood samples were withdrawn by heart puncture at timed intervals and liver was excised at the end of the experiment. ICG concentration in bile, plasma and liver were determined by a spectrophotometric method. To determine the transport maximum of ICG, guinea pigs were injected i.v. with a high initial priming dose and then the dye level in the plasma was maintained by constant infusion. Concentration of ICG in bile was determined periodically until a maximum was reached.

MAJOR FINDINGS: Among different age groups, much higher concentrations of ICG in bile were found in adults than in newborns, however there was no significant difference in bile flow. Less than 20 percent of the total administered dose

was excreted during a 20-minute period by newborn as compared to about 50 percent of the total dose excreted by adult. There was no significant difference between male and female animals of the same ages. The mean bile/plasma ICG concentration ratio in adults was approximately 50, while the newborn mean ratio was 7. Clearance from plasma to bile was greater than 50 ml/20 min for the adult while less than 2 ml/20 min for the newborn. Levels of maximal biliary transport capacity (Tm) was 275 and 85 µg/min/kg for adult and newborn respectively. Significant differences also existed in the plasma disappearance rates for the newborn and adult animals.

SIGNIFICANCE TO-BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Biotransformation, storage and excretion are the three major mechanisms by which environmental agents (including drugs) are ultimately eliminated from the body and their biological action terminated. Inefficient excretion in early postnatal life might be a hold-over from the period of intrauterine life and may account for more frequent accumulation of toxic compounds in the newborn. Our study increases knowledge on the process of biliary excretory development and this information should be useful in further study of the interaction of environmental agents on the developmental process.

PROPOSED COURSE: The effect of various environmental agents on the development of the biliary excretory process will be investigated.

Serial No.: NIEHS-PT-041
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effect of Age on the Binding of Indocyanine Green to Plasma, Bile and Liver Tissue of Guinea Pigs"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: S. W. Hwang, Ph.D.

OTHER INVESTIGATOR: R. L. Dixon, Ph.D. (Pathologic Physiology Branch)

COOPERATING UNITS: None

MAN YEARS:

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: It has been demonstrated that newborn guinea pigs have a lower capacity than adult guinea pigs to excrete indocyanine green (ICG) in the bile. The binding of ICG to plasma protein, bile protein and liver tissue may play a role in the overall excretory process. Therefore, the present study was designed to correlate the age-dependent ICG biliary excretion with the differences of the binding in plasma, bile and liver tissue of animals with various ages.

METHODS EMPLOYED: ICG binding to plasma or bile protein was studied by the Sephadex gel filtration method. For studying ICG binding in the liver tissue, an ultracentrifugation method was used. To study the in vitro uptake of ICG by liver slices, slices were prepared with a Stadie-Riggs microtome and incubated in Krebs-Ringer phosphate solution (pH 7.4) containing 1g glucose/l. The procedure of studying the runout of ICG from liver slices included first loading the slice with ICG and then transferring the slices through series of fresh medium. The ICG released into the medium and that remaining in the slices were determined.

MAJOR FINDINGS: ICG at 1×10^{-4}M was almost totally bound in the plasma of adult and newborn animals. Increasing the concentration decreased the percent bound. Adult plasma and newborn plasma showed no significant differences in ICG binding. The percent binding in bile for ICG added in vitro was the

same as that for ICG excreted in vivo. The newborn bile which contained higher protein showed slightly higher ICG binding than the adult plasma at both low and high ICG concentrations. For ICG binding in liver, 40 percent liver homogenate was tested at three different ICG concentrations. Adult liver had a higher ICG binding capacity at all concentrations used. The capacity of liver slices to take up ICG in vitro was compared. The slice to medium ratio increased with days after birth; however, S/M ratio for adult animals was lower than the S/M ratio for the 10-day-old animal which showed the peak S/M value. By comparing the runout rate of ICG among guinea pigs of different ages, it was apparent that the runout rate by adult liver was > 10-day-old > 1-day-old. ICG uptake and runout both appeared to mature with age. However, the runout rate must increase more rapidly than the uptake rate especially after 10 days of age because the adult had a smaller liver slice to medium ratio of ICG concentration.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The binding of a chemical to plasma, bile and liver tissue may directly contribute to the overall biliary excretion process. Clear knowledge about the binding processes and the stages of their development should increase the understanding of the hepatobiliary excretion process. Influence on the binding processes by environmental agents may be manifested as an overall decrease or increase of biliary excretion, so the correlation between binding properties and the overall excretory process may help clarify the whole process of biliary excretion and mechanism of action of some agents.

PROPOSED COURSE: Effects of environmental agents on the binding processes will be studied.

Serial No.: NIEHS-PT-042
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effect of Methylmercury on Hepatobiliary Function"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: S. W. Hwang, Ph.D.

OTHER INVESTIGATOR: M. Clemons

COOPERATING UNITS: None

MAN YEARS:

Total:	0.20
Professional:	0.10
Other:	0.10

PROJECT DESCRIPTION

OBJECTIVES: Although the major symptom of methylmercury toxicity is primarily neurological in nature, methylmercury is also concentrated in liver by an active process and damage to the liver tissue may result. It is the purpose of this investigation to determine the acute effect of i.p. injected methylmercury hydroxide (MMH) on liver function and bile excretion in guinea pigs.

METHODS EMPLOYED: Male guinea pigs were administered 5 mg/kg of MMH, i.p., for two consecutive days and controls received saline injections on the same time schedule. One, two or three days after MMH treatment, bile ducts of guinea pigs were cannulated. Animals were either injected with 10 mg/kg of bilirubin or 2 mg/kg of indocyanine green (ICG) and the biliary excretion of these compounds was observed. Activities of serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase were determined also to assess the functional state of the liver.

MAJOR FINDINGS: Animals loaded with injected exogenous bilirubin excreted less of both conjugated and unconjugated bilirubin after MMH treatment. Without exogenous bilirubin injection, the concentration of both conjugated and and unconjugated bilirubin in bile was increased after MMH treatment. Bile flow and biliary excretion of ICG were both significantly reduced after MMH treatment. Activities of SGPT and alkaline phosphatase in serum increased after MMH treatment suggesting hepatic damage. SGPT was elevated through the third day but the alkaline phosphatase activity in serum returned to normal by

the third day. The biliary alkaline phosphatase decreased 24 hours after MMH treatment and gradually returned to normal on days 2 and 3. Protein concentration in bile increased after MMH treatment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mercury contamination in the environment has caused several crises in public health. In order to evaluate the extent of mercury hazard to animals and man, information is needed on how mercury damages specific organs and tissue. The present study provides information on how organic mercury affects liver function.

PROPOSED COURSE: We propose studying differences between acute effects of MMH with chronic effects after long-term exposure to low doses of MMH.

Serial No.: NIEHS-PT-043
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Effect of TCDD on the Biliary Excretion of ICG"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: S. W. Hwang, Ph.D.

OTHER INVESTIGATOR: M. Clemons

COOPERATING UNITS: None

MAN YEARS:

Total:	0.20
Professional:	0.10
Other:	0.10

PROJECT DESCRIPTION

OBJECTIVES: Chlorinated dibenzo-p-dioxins, as contaminants, may be responsible for disease processes in animals associated with the exposure to chlorinated technical compounds such as PCB and 2,4,5,-T. The objective of the present study was to observe directly the effect of 2,3,6,7-tetrachlorodibenzo-p-dioxin (TCDD) on hepatic function using indocyanine green (ICG) excretion as an indicator.

METHODS EMPLOYED: Rats were treated p.o. with a single dose of 25 μg/kg or 5 μg/kg TCDD. At days 1, 7, and 16 after treatment, animals were anesthetized and bile ducts cannulated. Freshly prepared ICG solution was injected i.v. and 20 minutes after injection, ICG concentrations in bile, plasma and liver were determined by spectrophotometric methods.

MAJOR FINDINGS: The flow rate of bile increased after TCDD treatment and continued to increase without showing recovery up to 16 days after treatment. The total amount of ICG excreted during 20 minutes after injection of the dye (6.25 mg/kg) was decreased significantly and no sign of recovery was shown by 16 days after TCDD treatment. The ICG levels in plasma and liver of TCDD treated rats were higher than those in control rats. Animals dosed with 25 mg/kg of TCDD showed a greater depression of ICG concentration in bile and greater retention of ICG in plasma. In contrast, low-dosed animals accumulated more ICG in liver than the high-dosed animals. The rate of ICG disappearance from plasma decreased after TCDD treatment. Liver weights of the rats increased after TCDD treatment.

SIGNIFICANCE TO BIO MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of the present study indicated that TCDD treatment significantly retarded liver function for excretion of ICG. The decreased excretory ability might apply also to other compounds and would possibly result in the accumulation of metabolites or toxic compounds in the body. This project is part of the TCDD studies in which other laboratories of NIEHS participated.

PROPOSED COURSE: This project will be completed in the near future.

Serial No.: NIEHS-PT-044
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Hexachlorobenzene"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-035

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: H. B. Matthews, Ph.D., R. L. Metcalf, Ph.D., and L. F. Fishbein, Ph.D.

COOPERATING UNITS: University of Illinois

MAN YEARS:

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES:

1. To study metabolism, storage and excretion of hexachlorobenzene in rats and the effects of pretreatment with HCB on hepatic microsomal enzymes and cytochromes.

2. To study metabolism and fate of HCB in a model ecosystem.

METHODS EMPLOYED: A single radioactive dose of HCB such as might be encountered in the environment was administered to male rats. In addition, *in vitro* metabolism by rat liver preparations was investigated.

The model ecosystem studies included an interaction of terrestrial and water interphases as follows: (1) the terrestrial interphase included corn plants on which the compound was applied, (2) insect caterpillars which fed on the plants, (3) insect excreta introduced the compound to the water phase of the model ecosystem, and (4) a food chain consisting of snails, water crustaceans, mosquito larvae, algae and fish was exposed to the compound in the water phase. The metabolic fate and bio-accumulation of the toxic agent was studied at the organismic level and in the food chain as a whole.

MAJOR FINDINGS: HCB-^{14}C was administered to a group of 5 male rats as a single oral dose (5 mg/kg) in arachis oil. Fecal excretion of HCB was less than

20 percent while urinary excretion was less than 1 percent of the total dose over a 7-day period. HCB was stored extensively in the body tissues, fat having the highest concentration. Examination of the excretion pattern of HCB in feces and urine for 7 days after administration indicated that excretion totally ceased by 3 to 4 weeks with over 90 percent of tissue stores found after 7 days still retained in the body. The most complete pattern of metabolism of HCB was obtained from the urine. Pentachlorophenol, pentachlorobenzene tetrachlorobenzene, and 2,4,5-trichlorophenol have been tentatively identified as urinary metabolites. Every tissue examined also had chlorobenzene metabolites. Rats receiving oral doses of HCB, 25 mg/kg, for 6 days had slightly enlarged livers with no change in body weight. There was about a 40 percent increase in hepatic cytochrome P450 and only 12 percent increase in cytochrome b_5. Aniline hydroxylase and N-demethylase activities were increased. Glucuronidation of I-naphthol was increased close to three-fold in the treated rats. Coproporphyrin in urine was increased five-fold by HCB treatment. Pre-exposure to HCB had slight effects on fecal excretion of HCB-^{14}C. Rats pretreated for 6 days with HCB excreted in the feces about 28 percent more of a single dose of HCB-^{14}C than control animals. Urinary excretion was unaffected. In vitro incubation of HCB with rat liver, lung, kidney and intestine indicated that these tissues all actively metabolized HCB, producting patterns of metabolism similar to those in the urine.

Studies with the model ecosystem indicated that relatively large quantities of HCB were taken up by algae, daphnia, mosquito larvae and fish. A 30-day study of the model ecosystem after application of 5 mg HCB to sorghum plants indicated that fish and snails retain large amounts (1000 x the water concentrations) of radioactive HCB. Examination of extracts of salt-marsh caterpillar and other components of the ecosystem indicated that various organisms differed substantially in their ability to remove chlorine from HCB.

SIGNIFICANCE TO BIO-MEDIAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: HCB is a common fungicide used as a seed-dressing to protect seeds from various fungal deseases. It is also an important industrial pollutant because it is synthesized during non-specific large-scale chlorination of aromatics. It is known to cause porphyria cutanea tarda in human beings. The effects can last beyond exposure to the compound. An understanding of the metabolism, storage, and excretion patterns might be helpful in understanding the mechanism of porphyria induction.

PROPOSED COURSE: The project has been terminated.

Serial No.: NIEHS-PT-045
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Aldrin Metabolism by Plant Root Preparations"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-041

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.6
Professional:	0.6
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Qualitative assessment of the nature of the polar metabolites of aldrin in plant root systems. To obtain mass spectroscopic evidence for the presence of cis- and trans-aldrindiols.

METHODS EMPLOYED: Aldrin was incubated with active bean (Phaseolus vulgaris) and pea (Pisum sativum) root preparations. Metabolites were extracted and analyzed by various chromatographic techniques. Final purified preparations were subjected to mass-spectroscopy for confirmation of identity. Various compounds known to be intermediates of aldrin or other organisms were incubated with plant root preparations in an attempt to establish metabolic pathways for the formation of polar metabolites.

MAJOR FINDINGS: Gas chromatographic analyses of the products of aldrin incubations with bean and pea root preparations indicated the presence of a series of related polar metabolites other than dieldrin of which trans- and cis-aldrin diols and aldrin ketone appear to be predominant. Mass spectral analyses of silylated incubation products have confirmed the presence of both the aldrin diols in nearly equal quantities albeit in low yields based on starting substrate along with aldrin alcohol and aldrin ketone. Incubation of dieldrin, dihydroaldrin, exo-aldrin alcohol, cis-aldrin diol and aldrin ketone at two incubation concentrations with active root preparations did not show any metabolism. Traces of aldrin ketone were detected - with exo-aldrin alcohol incubations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aldrin is a major chlorinated cyclodiene pesticide and is a common environmental contaminant. While its fate in almost all target and non-target organisms is fairly well understood, the metabolism in plants has not been fully elaborated. The nature of the residues one encounters in the environment depends partly on the metabolic transformation that aldrin undergoes in plants. Thus, an understanding of the pathways of metabolism to which environmental agents of this nature may be subjected in plants would be valuable.

PROPOSED COURSE: The project is being terminated as other areas of the program need more attention.

PUBLICATIONS

Mehendale, H. M.: Aldrin epoxidation by plant root preparations. Phytochemistry (in press).

McKinney, J. D., dePaul Palaszek, S. M., Matthews, H. B., and Mehendale, H. M.: Stereochemical aspects of the metabolism and toxicity of some chlorinated polycyclodiene pesticide systems. Industrial Medicine and Surgery. 1973 (in press).

Serial No.: NIEHS-PT-046
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Saccharin"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-028

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATOR: M. Fields

COOPERATING UNITS: None

MAN YEARS:

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the in vivo and in vitro metabolism of saccharin and to investigate the distribution, storage and excretion of a small dose of saccharin in the rat.

METHODS EMPLOYED: The studies have followed in vivo and in vitro metabolism, utilizing radiotracer techniques, biological sample oxidation, liquid scintillation counting, various chromatographic techniques and mass spectral analysis.

MAJOR FINDINGS: A dose of saccharin (1 mg/kg), which represents 20 percent of the recommended "safe" dose was found to be rapidly absorbed from the gastrointestinal tract, distributed throughout the body and excreted primarily in the urine. Blood and tissue levels reached peak concentrations within 15 minutes after saccharin administration and decreased thereafter. The magnitude of the peak concentrations were dependent upon whether the animals had been fed or starved prior to saccharin administration. Saccharin was rapidly cleared from most tissues; however, administration of multiple doses resulted in accumulation of saccharin in some tissues, particularly the urinary bladder. Removal of saccharin from the diet resulted in almost complete tissue clearance within 3 days. No metabolism of saccharin was detected in either in vivo or in vitro studies.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possibility of potentially harmful metabolites of saccharin, e.g., N-hydroxy

and related derivatives, had not been ruled out by previous studies. Also, tissue distribution and storage of a low dose of saccharin had never been investigated.

PROPOSED COURSE: The study has been completed and a report of the results has been submitted for publication.

Serial No.: NIEHS-PT-047
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Metabolism of Dieldrin"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-024

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: J. D. McKinney, Ph.D., and M. Fields

COOPERATING UNITS: None

MAN YEARS:

Total:	0.8
Professional:	0.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: To study the metabolism, storage and rate of excretion of dieldrin in the rat following the administration of doses possibly analogous to that resulting from an environmental exposure (e.g., a single 10 ppm oral administration). To investigate the enzyme mechanisms involved in the metabolism of dieldrin.

METHODS EMPLOYED: Whole animal and enzymatic metabolism studies, thin-layer and gas chromatography, isotope dilution, NMR spectroscopy and mass spectral analysis have been used to produce, isolate and identify the metabolites of dieldrin.

MAJOR FINDINGS: Trans-aldrindiol has been shown to be a metabolite of dieldrin in these and other studies. The cis isomer of the diol however has not been reported as a metabolite of dieldrin despite the fact that the stereochemical structure of dieldrin is such that the formation of the cis isomer should be more ordered and energetically more favorable. Our studies have shown cis-aldrindiol to be an in vitro metabolite of dieldrin; however, it exists only in trace amounts at what appears to be a steady-state concentration, whereas the concentration of the trans isomer continues to increase over 1 hour's incubation time. Therefore, we investigated the in vitro metabolism of synthetic cis-aldrindiol, and have shown that this compound was rapidly converted to the trans-isomer by the same in vitro enzyme preparation which metabolizes dieldrin. Also, the product of this enzymatic epimerization has been shown to be optically active just as the trans-isomer isolated in vivo studies is optically active.

The enzyme responsible for the epimerization of the cis to the trans-aldrindiol has been shown to be located in the microsomes, to require a reduced nucleotide cofactor and to be inhibited by carbon monoxide. Thus, the enzyme appears to be like a typical mixed-function oxidase except that it is not inhibited by an anaerobic atmosphere and it is only slightly inhibited by insecticide synergists. On the other hand, the enzyme is quite sensitive to inhibition by metyrapone, a drug known to bind to cytochrome P450 in a typical manner.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Dieldrin is a "hard" pesticide and even though its use is declining, its existence in the environment will continue for some time; therefore, definitive knowledge of its metabolism is essential. Also, the unique stereochemical configuration and stability of the dieldrin molecule allowed us to study a basic biochemical reaction (epimerization) which would not have been possible with a compound having a less rigid structure.

PROPOSED COURSE: The study has been taken as far as planned for the present. The final results are currently being written up to be published.

PUBLICATIONS

McKinney, J. D, Matthews, H. B., and Fishbein, L.: The major fecal metabolite of dieldrin in the rat. Its structure and chemistry. J. Ag. Food Chem. 20(3): 597, 1972.

Serial No.: NIEHS-PT-048
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effect of Mirex Pretreatment on Rat Liver and Its Microsomal Mixed-Function Oxidase System"

PREVIOUS SERIAL NUMBER: NIEHS-ACS-042

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: P. R. Chen, Ph.D., H. B. Matthews, Ph.D., and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To study the effect of orally administered daily doses of Mirex (e.g., 5 mg/kg) on male and female rat liver and liver microsomal enzymes.

METHODS EMPLOYED: Animals were treated daily for 5 days and sacrificed on the seventh day. Liver and body weight were measured and the enzyme activities were determined: (a) N-demethylation of ethylmorphine, (b) O-demethylation of p-nitroanisole, (c) aromatic hydroxylation using aniline as a substrate, and (d) glucuronyl transferase using 1-naphthol as substrate.

MAJOR FINDINGS: Normal body weight gain was significantly reduced in rats of both sexes at 5, 10, 25, and 50 mg/kg of Mirex. At 25 and 50 mg/kg, female rats actually lost weight, and at 50 mg/kg, there were some deaths. Liver weight increased in all animals in proportion to increase in dosage, and liver to body weight ratios were doubled in both sexes at 25 mg/kg.

Activities for the O-demethylation of p-nitroanisole and N-demethylation of ethylmorphine were increased after the lower doses of Mirex while there was no change in total activity at the higher doses. Aniline hydroxylase activity was decreased in both sexes at all doses, and this effect apparently was not due to binding of Mirex to the microsomes. There was an increase in the total glucuronyl transferase activity for conjugation of 1-naphthol but not in the specific activity. Mirex induces the synthesis of P450 with increased Type II binding

characteristics while inhibiting the metabolism of at least one Type II substrate (aniline).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mirex is one of the most persistent pesticides known and will be ingested by man as a result of environmental exposure. An evaluation of its effects on the biochemistry of the liver is desirable in order to more fully understand the implications of its hazard as an environmental pollutant.

PROPOSED COURSE: This project is temporarily suspended in favor of other projects with the Pharmacokinetics Section.

Serial No.: NIEHS-PT-049
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "In Vitro Metabolism of PCB"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-037

PRINCIPAL INVESTIGATOR: P. R. Chen, Ph.D.

OTHER INVESTIGATOR: L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.6
Professional:	0.5
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To study the mode of action and metabolic fate of a variety of isomeric PCB's.

METHODS EMPLOYED: Chromatography (TLC, column, and GLC), NMR mass spectroscopy. Carbon-14 labeled biphenyl and monochlorobiphenyls (2-,3-, and 4-chlorobiphenyls) were incubated with rat hepatic microsomes and soluble fraction fortified with NADPH, then extracted with chloroform.

MAJOR FINDINGS: Four chloroform soluble metabolites were separated by TLC in three different solvent systems and tentatively identified as the corresponding 2'-hydroxyl-, 4'-hydroxyl, 3',4'-dihydroxyl and 4,4'-dihydroxyl products. The final structures will be verified by NMR and mass spectroscopy. The aqueous layers remaining after solvent extraction were passed through Sephadex G-25 columns with three radioactive peaks found in the eluate. The first of these eluted off with protein in the void volume from a Sephadex G-75 column, suggesting it was a protein-bound compound. The other metabolites were identified as glucuronides because biosynthesized glucuronides eluted off in the same fractions from both Sephadex G-25 and G-75 columns.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the biotransformation and the identification of the metabolites of the ubiquitous and environmentally important PCB's is essential in understanding their mode of action and potential hazard to environmental health.

PROPOSED COURSE: Epoxides have been found to be metabolites of several polycyclic hydrocarbons because they yield various phenolic products on rearrangement under different conditions. They also have been shown to be cytotoxic to cell culture due to their ability to bind DNA, RNA and protein. PCB's also are aromatic hydrocarbons. Thus, efforts will be made to determine whether epoxides are involved in the biotransformation of PCB's. Identification of other unknown metabolite(s) will be attempted.

Serial No.: NIEHS-PT-050
1. Pharmacology and Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Induction of Hepatic Microsomal Enzymes by Two Tetrachlorobiphenyls"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-038

PRINCIPAL INVESTIGATOR: P. R. Chen, Ph.D.

OTHER INVESTIGATORS: H. M. Mehendale, Ph.D., and L. Fishbein, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.6
Professional:	0.4
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the biochemical effects of several isomeric tetrachlorobiphenyls in the rat.

METHODS EMPLOYED: Enzyme assay, thin-layer and radio-chromatography. Induction of hepatic microsomal enzyme by two tetrachlorobiphenyls.

MAJOR FINDINGS: Oral administration of two isomeric tetrachlorobiphenyls (TCB's) (2,3,4,5- and 3,5,3',5'-tetrachlorobiphenyls) to rats at three dosage levels does not cause any adverse effect on their growth. However, several hepatic enzyme systems are affected, even at the lowest level (5 mg/kg). Ethylmorphine N-demethylase and aniline hydroxylase activities are slightly induced by 2,3,4,5-TCB but, at a medium dose level (20 mg/kg), the activity of para-nitroanisole O-demethylase is induced two-fold. Also, 3,5,3',5'-TCB induces these enzyme systems in male rats, but decreases the activities of aniline hydroxylase and ethylmorphine N-demethylase in female rats at higher doses. An "extra" polar metabolite occurs in the in vitro metabolism of oestradiol-^{3}H when female rats are treated with 2,3,4,5'-TCB at dose level of 40 mg/kg. UDP-glucuronyl-transferase and δ-aminolevulinic acid synthetase activities were not affected by the administration of these two TCB's.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elaboration of the effects of specific purified isomers of the polychlorinated biphenyls on various hepatic enzymes is essential to the assessment of the hazard of this important class of ubiquitous environmental agents.

PROPOSED COURSE: This study was terminated,because PCB's did not cause obvious adverse effects other than hepatic enzyme induction.

PUBLICATIONS

Chen, P. R.: Effect of two isomeric tetrachlorobiphenyls on rats and their hepatic enzymes. Archives of Environ. Contamination and Toxicol., (in press).

RESEARCH SERVICES BRANCH

RESEARCH SERVICES BRANCH
Summary Statement

The Research Services Branch provides support services to the Institute in the form of planning and acquiring appropriate facilities; operating, altering and maintaining the facilities; providing sterile glassware and media for the laboratories; providing a safety and security program; designing, fabricating and maintaining research associated instrumentation; and conducting research in the broad areas of noise and ionizing and non-ionizing radiation.

The Office of Management and Budget apportioned $2,000,000 in May, 1973 in addition to the $1,667,500 previously apportioned planning monies for the NIEHS permanent facilities. The Facilities Engineering and Construction Agency, DHEW is expected to select an architectural/engineering firm soon to initiate design of this $50 million facility. The facility will be located on the 509 acre tract immediately east and south of the interim facilities in the Research Triangle Park. Design will take approximately two years. The Department is proposing that construction funding be acquired through lease-purchase rather than direct federal construction. Plans are to retain the interim facilities beyond occupancy of the permanent facilities.

A contract was signed in May, 1973 for construction of a 9,600 square foot office building to be located at the north end of the NIEHS interim site. Occupancy is expected in the third quarter of FY 1974.

The warehouse function has been moved some 4 miles from the NIEHS site into leased space. The on-site warehouse building is currently being converted into light laboratory and support/service space. Renovations are scheduled to be completed in the first quarter of FY 1974.

Laboratory requirements for glassware and media have increased by 50% in the first nine months of FY 1973 over the same time period in FY 1972. Activation of the mutagenesis program has contributed substantially to the increases.

The following are highlights of research and events for the Biophysics and Instrumentation Section during FY 1973.

Noise

A sound and electromagnetically isolated facility for noise exposure and electrophysiology was designed, procured, installed and tested for conformance with specifications. A noise generation system was installed and tested. This system has the capability of maintaining white noise levels of 125 dB (20-20 KHz) indefinitely and over an area sufficient for exposure of approximately 20 guinea pigs simultaneously.

In a series of experiments carried out in the room, it was found that young guinea pigs suffered significantly more intracochlear permanent damage from noise exposure than did mature guinea pigs exposed to the same noise. Examination of the noise exposed inner ear structures was carried out by means of phase contrast microscopy. Other experiments were designed and carried out to measure the combined effects of noise exposure and sodium salicylate.

In another experiment, the effect of methyl mercury upon the cochlear structures was found to predominate in the apical rather than the basal region making it a unique ototoxic agent.

Evaluation of the results of an experiment where guinea pigs were exposed to the combined effects of ethacrynic acid and salicylates is in progress. In the non-auditory effects area, the influence of pre-exposure of rats to moderate to high noise levels was found to affect hexobarbitol sleeping times (drug metabolism) in a preliminary study. In the very near future, we intend to measure various cardiac parameters in rabbits under the influence of acute noise stress.

A flexure mode piezoelectric transducer for direct driving of the ossicular chain of research animals was developed and tested. Since the magnitude of the movement of the ossicular chain in the audio frequency range is only on the order of Angstroms (10^{-10} meters) a new light lever technique had to be adapted to make the measurements. Ossicular chain displacements for various sound pressure levels and frequencies were measured. This was the first time such measurements had been made without use of an enormously expensive laser inferometer.

A contract was let to the Kresge Hearing Institute to use the synergism present between noise and ototoxic drugs to study mechanisms of hearing loss at the biochemical level. Present work is centered on disruption of membrane (lipid) metabolism. This work is one of the few attempts being made worldwide to study this enormously difficult area.

Staff members are serving as DHEW representatives on the EPA task force working on methods of characterizing airport/aircraft noise levels with respect to available technology and public health and welfare. This EPA effort was mandated by the Noise Control Act of 1972.

Dr Stephen Falk authored a review article "Combined Effects of Noise and Ototoxic Drugs."

Dr. Stephen Falk was asked to write an article on the effects of noise on the auditory organ for the Handbook of Physiology.

Mr. Reginald Cook was elected President of the North Carolina Acoustical Society.

Radiation

An X-ray emission spectrometry system has been developed for the determination of trace elements in biological samples. The system is applicable to elements

with atomic number greater than 12. A minimum detectability for mercury of 2 micrograms in 100 seconds has been demonstrated. An improved system offers substantially lower minimum detectabilities.

A microwave dosimetry system based upon energy absorption measurement has been developed. The system can be described using simple physical principles and thus can be used as a reference for incident power density measuring devices in attempting to relate field intensities and frequency to actual energy absorption.

Theoretical dose-response relationships have been developed for cell alteration and cell survival. The model considers the relationships among such parameters as cell turnover time, dose rate, cell repair and division of altered cells. The model allows for comparison with experimental data where it exists and extrapolation to low dose-rate, low dose conditions where data is not available.

Studies on airborn hazards have reemphasized information gaps. These include determination of dose to respiratory tract tissue from inhalation of a toxic material, the relevance of dose patterns and extrapolation of data between animal species and to man. To aid in obtaining better information, studies are supported at Lovelace Foundation that will determine the deposition of various-sized aerosols in the respiratory tracts of man and several animal species. The objectives of the research are:

a) Construction of physical and mathematical models of the respiratory tracts of man and several animal species;

b) Measurement of the deposition of aerosols as small as 0.01 micron diameter in the models;

c) Determination of deposition of selected particle sizes in animals and in man, as is practical;

d) Development of theoretical models for calculating regional deposition in the lungs based on the above results;

e) Field evaluation of the physical-theoretical model.

Progress has been made on objectives a, b and d. Silastic casts of the lungs of several animal species including man have been made. Morphometric measurements on these casts have classified branches of tracheobronchial trees according to length, diameter, branching angle, angle to gravity and any unusual characteristics. Each branch is numbered according to a novel ordering system that can be used to relocate and remeasure any particular branch region. An algorithm especially suited to tree-like models has been used to generate a computer probabilistic model of the lung based upon the ordered morphometric data. A computer calculation of deposition in this model can be accomplished using any improved mathematical formulation with the experimental deposition data being accumulated.

Microwaves

Techniques for measuring the amount of microwave energy absorbed by biological specimens were studied. Thermocouple and small thermistor probes were evaluated, and small thermistors which do not interfere with absorption were adapted for use. Analytical techniques for determining time-temperature profiles and energy absorption were derived from the absorption rate and cooling curve data from these thermistors. An infrared microscope was investigated to determine its accuracy and utility in remote monitoring of specimen temperature during exposure. This technique proved accurate to within ±1/2°C and will be used in various applications. Liquid crystals were evaluated as a temperature indicator on the basis that color refraction varies with temperature. In some applications, they will be useful and quantitative to within 1°C. Pyroelectric detectors, which were developed under contract to Georgia Tech will be supplied by the end of June. These detectors will measure free field power density in the frequency range of 1-10 GHz.

Four strains of E-coli were exposed to 2450 MHz at power densities of 60 and 240 mW/cm^2. No change in replication was observed. DNA was exposed to 2450 MHz at power densities of 134 to 320 mW/cm^2. No effects on thermal denaturation of the DNA was obtained.

A pilot study on the exposure of fertilized quail eggs to 2450 MHz microwave radiation was carried out. Separate groups of 20 eggs were exposed to 30 mW/cm^2 power levels (level necessary to maintain the eggs in the field at the same temperature as the controls in the incubator, 37°C) for 4 hours each day for the first 7 days. The eggs were then taken to hatch which was 17 1/2 days. The eggs exposed at the 3 and 4 day stage had 2 times as many early dead embryos as the controls for these groups. Low blood levels and proliferated thymus were observed in the exposed birds which did hatch. Studies are now being planned to more closely analyze and verify these effects.

Specific factors in the design of experiments to study neural effects has been studied. This includes specification and ordering of equipment. Studies under contract at Duke University on microwave-neuron interactions indicate non-thermal effects on neural functioning. A decrease in firing rate and post synaptic activity were observed.

During the past year, Donald I. McRee was elected chairman of the Scientific Information Exchange Subgroup of IRAC, Interdepartment Radio Advisory Committee, of the Office of Telecommunications Policy. During the past year, the Planning Subgroup has been organized and a workshop on behavioral effects of microwaves was held on April 9-10. The workshop was attended by 13 scientists actually doing the work in this area. Another workshop for researchers is being planned for the fall.

Serial No.: NIEHS RSB-001
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Respiratory Tract Dose Model for Uranium Miners"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. J. Walsh, Ph.D.

OTHER INVESTIGATOR: D. I. McRee, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	1.3
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to develop a respiratory tract dose model that will provide reliable estimates of the dose of radiation to the basal cells of the bronchial epithelium of the respiratory tract in the case of inhalation of alpha emitters.

METHODS EMPLOYED: Calculation of the dose to the basal cells in each anatomical region of the respiratory tract requires a detailed description of the exposure atmosphere, a detailed respiratory tract model, accurate techniques of deposition calculation and an accurate analysis of the effects of clearance.

MAJOR FINDINGS: Work on this project was re-directed during fiscal year 1972. A new contract was initiated to develop a respiratory tract deposition model (see Lovelace Foundation), and research was begun on novel techniques for determination of deposition in the respiratory tract (see NIEHS-RSB-017). The publications listed below have been useful to a National Academy of Sciences Subcommittee on the Biological Effects of Ionizing Radiation and to a special working group of the International Commission on Radiation Protection on the utility of measuring working level concentrations or radon gas concentrations in uranium mines. Information on the dose calculations have been requested by representatives of NIOSH and EPA.

Detailed information needed to improve the models is being obtained through the contract with Lovelace Foundation and in NIEHS-RSB-002.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of an ideal respiratory tract dose model would require improved input information. This information would be crucial to the assessment of the risk associated with the possible inhalation of any toxic material. Such a model would have immediate applicability to the studies of the health hazards of uranium mining.

PROPOSED COURSE: Experimental measurement of the deposition of ions and particulates in models of the tracheobronchial tree is one example of information needed to develop a comprehensive deposition theory (see NIEHS-RSB-007 and Lovelace Foundation contract). The effects of the wall characteristics and eddy current at bifurcations will be important considerations. Additional physiological and biological information is also needed. The respiratory tract dose model will be elaborated in accord with availability of basic input information.

PUBLICATIONS

Walsh, P. J.: Radiation dose to the respiratory tract of uranium miners - A review of the literature. Environmental Research 3:14-36, 1970.

Walsh, P. J. and McRee, D. I.: Depth-dose curves for alpha particles from area sources and point sources. Health Physics 20:352, 1971.

Gastineau, R. M. and Walsh, P. J.: Thickness of bronchial epithelium with relation to exposure to radon. Health Physics 23:6, 1972.

Serial No.: NIEHS-RSB-002
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Energy Loss Characteristics of Radiation in Matter"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. J. Walsh, Ph.D.

OTHER INVESTIGATORS: P. Hamrick, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.6
Other:	0.9

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to obtain more information on the main interactions of ionizing radiation with matter, such as ionization and excitation of atoms and molecules, and the more subtle interactions of radiation with molecules, such as the effect of the penetrating radiation upon molecular bonds. Such information will be basic to further analysis of the connections between physical energy loss and absorption processes and biological effects.

METHODS EMPLOYED: The measurement of the energy loss of radiation is accomplished by using a solid state radiation spectrometer. The complete spectrometer consists of a measurement chamber containing an alpha, beta or gamma radiation source, multiple samples of the material to be studied and a solid state radiation detector. The doses and dose rates of alpha, beta and gamma radiation can be varied at will by adjusting the source-absorber geometry; and the solid state spectrometer allows an accurate determination of the energy loss in the absorber at the same time. This provides an experimental check on theoretical dose estimation. Biological absorbers can be studied with this system.

MAJOR FINDINGS: During FY 1972 a theoretical model was developed which described the effect of dose-rate and turnover time of cells on dose-response relationships. The model has been extended to include the possible effects of division and repair of altered cells on dose-response relationships. From

the model, speculation on the nature of dose response relationships for cancer was possible.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Further information on the nature of interactions of ionizing radiation with matter is necessary for more accurate dose calculation and for improving our understanding of the connections between damage to atoms and molecules and subsequent biological effects. Effort must be applied to the development of realistic models since it may be impossible to obtain sufficient experimental data especially under low dose conditions.

PROPOSED COURSE: In addition to, and as an aid to model development, the project can be expanded to:

1. Study the energy loss characteristics of radiation in cell suspensions and cell cultures and develop quantitative dosimetric concepts which descripe the results;

2. Deliver known doses of radiation to cells at various dose rates to determin dose-response relationships as a function of dose rate and turnover time of the cells;

3. Study the relative effectiveness of alpha, beta and gamma radiation for a given end point;

4. Use chemical or biological toxicants or protectors in conjunction with the radiation. Such studies should identify the most important molecular components in terms of biological response.

PUBLICATIONS

Walsh, P. J.: On the application of the uncertainty principle to stopping power theories. Health Physics 19:574, 1970.

Walsh, P. J.: Stopping power and range of alpha particles. Health Physics 19:312-316, 1970.

Walsh, P. J.: Possible effects of dose rate and turnover time of cells on dose response relationships: implications for radiation protection guidance. International Journal of Environmental Studies 2:189-193, 1971.

Walsh, P. J.: Energy loss of alpha particles in tissue equivalent plastic. Health Physics 23:701-704, 1972.

Serial No.: NIEHS-RSB-003
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Services Branch

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Microwave Absorption by Biological Material"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: D. I. McRee, Ph.D.

OTHER INVESTIGATOR: Philip E. Hamrick, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine the absorption of microwave radiation by biological material, to investigate the contribution of cell structure to the absorption and to attempt to explain the mechanism of interaction between microwaves and the biological material.

METHODS EMPLOYED: DNA, RNA, protein, cell cultures and biologically simulated material (alcohol-water solutions) will be exposed to 2450 MHz microwave radiation. The amount of energy absorption will be measured, and the changes in the media will be evaluated.

MAJOR FINDINGS: Studies have been made which tend to suggest that non-thermal microwave fields at various frequencies interact with intracellular moieties to alter metabolic and/or genetic processes. Interpretation of these effects is difficult because mechanisms are not specified. The absorption of microwave energy by RNA, DNA, protein and complete cells appears to be frequency dependent. It is not known whether the absorption of 2450 MHz microwave radiation is different in the above media. With the establishment of our variable frequency system, absorption at other frequencies will also be determined. Mouse lymphoma cells were exposed to 2450 MHz radiation. The cells were exposed at a power level of 90 mW/cm^2. From model experiments, it was determined that an equilibrium temperature of 37°C. could be maintained at this power level. The cells were exposed for 1 hour, 2 hour and 4 hour durations with specimens taken at the end of each exposure period. Tests for mutagenic effects

at two particular loci within the cell were made. These tests showed no effects.

Most of the effort this past year was concentrated on evaluating possible techniques for accurately measuring the absorbed energy. Preliminary results indicate that thermistor probes are presently the best available technique for use with our exposure system. This thermistor was used to measure the time-temperature profiles of 5 ml of distilled water in a plastic test tube while being exposed to various power density fields of microwave radiation. Using this data and basic thermodynamic principles, a mathematical model was developed which agreed with 1°C. with the experimental value. Because there is some concern over the presence of the thermistor in the specimen while the microwave field is being emitted, a mathematical model was also developed from the cooling curve which provides the total time-temperature profile for the specimen. These mathematical models enable an investigator to evaluate what power level of exposure he wishes to use to maintain a certain temperature during irradiation, to obtain the total time-temperature profiles for any power density level so that he may subject his controls to the same time-temperature profile, and to obtain the dose of microwave radiation absorbed by the specimen.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanisms of interaction of microwave radiation and biological material need to be specified before an accurate evaluation of effects can be obtained. Many investigators believe that all effects, so far observed, are thermal effects. However, some studies have shown effects which were not due to a rise in temperature. More basic information about the interaction mechanisms will assist in the interpretation of these non-thermal effects.

PROPOSED COURSE: Studies on the absorption of microwave radiation by simulated biological material will be performed in order to obtain a measure of the electrical properties of biological specimens. Cell components such as RNA, DNA and protein, as well as the cells themselves, will be exposed, and the energy absorption measured. By studying the absorption of particular molecules which make up the cell, an insight into the mechanism of interaction will be obtained. Because the absorption of microwave radiation by a specimen is dependent upon its electrical properties, it is important to have the capability of measuring these properties of a biological specimen. This capability will be developed during the coming year.

PUBLICATIONS

McRee, D. I.: Environmental aspects of microwave radiation. Environmental Health Perspectives, Exp. Vol. 2, Oct. 1972.

McRee, D. I.: Biological effects of microwave radiation. The Proceedings of the Air Pollution Control Association Journal. (In Press)

Serial No.: NIEHS-RSB-005
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Microwave Exposure System and Microwave Dosimetry"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATORS: P. J. Walsh, Ph.D. and R. H. Wyatt, Ph.D.

COOPERATING UNIT: Georgia Institute of Technology, Engineering Experimental Station, Electronics Division

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to develop a microwave exposure system for biological material which has a well-defined, uniform field characteristic; and to develop dosimetric relationships for energy absorption.

METHODS EMPLOYED: A thorough study of the research requirements on the biological effects of microwaves was completed. Based on these studies, a set of specifications was written for a microwave exposure system which would generate a uniform, low-level microwave field in a small laboratory space. At present, it is difficult to accurately measure low level microwave fields and to determine the mechanisms by which the fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, chemical reactions, liquid crystals and photographic film are some of the technics which will be investigated.

MAJOR FINDINGS: A microwave exposure system with a frequency of 2450 MHz has been designed, built and the field calibrated. The microwave field at the specimen location was determined to be uniform within the design specifications with variation in power density as follows:

6 inch diameter area - ±5%
9 inch diameter area - ±10%
12 inch diameter area - ±15%

The system provides a power density range of 0.01 mW/cm^2 to 200 mW/cm^2.

Thermistors and thermocouples have been evaluated for interaction with the microwave field. The thermistor and thermocouples were placed in the field with only air as the surrounding medium. The thermistor was not affected whether the leads to the thermistor bead were or were not shielded and/or grounded. The thermocouple was very sensitive to microwave interference if the leads were not grounded. Even when grounded some instability was noted. In future absorption and biological effects research, the thermistor will be used to monitor temperature.

An analytical model for the power-time thresholds for lenticular damage has been developed. The absorption of energy in the eye results in a rise in temperature of the fluid in the lens. By determining the absorption and dissipation rate constants of the lens from existing data in the literature, an analytical model was developed for power-time thresholds for cataractogenesis.

An infrared microscope for measuring the temperature of exposed specimens remotely has been evaluated. The infrared microscope proved to be an extremely useful instrument for measuring the time-temperature profiles of a body being exposed to microwave radiation without the necessity of having a probe in the body or other material in the field. Liquid crystal detection of temperature was also studied. Although this technique is not as quantitative as other techniques, in some applications these liquid crystals are very useful in visually observing the temperature of a body while being exposed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The well defined microwave field will allow the Institute to perform accurate quantitative studies of the effects of microwave radiation in biological systems at a frequency of 2450 MHz. Using the thermistor to measure the temperature profile of a specimen during irradiation enables us to duplicate this temperature in the controls using ordinary heating techniques. This procedure will enable us to separate thermal and non-thermal effects. The analytical model for threshold levels for the development of eye cataracts enables interested scientists to determine safe exposure levels and provides a technique for extension to all frequencies as more experimental data become available.

PROPOSED COURSE: An infrared microscope will be obtained and will become a very useful instrument for measuring temperature of an exposed body. Liquid crystal detection will be evaluated in various applications and their accuracy determined. The pressure transducer-capillary detector which has been fabricated will be tested as to its accuracy, sensitivity, and response time. Implantible electric and magnetic field probes will be investigated as they become available.

PUBLICATIONS

McRee, D. I.: Thresholds for lenticular damage in the rabbit eye due to single exposure to CW microwave radiation: an analysis of the experimental information at a frequency of 2.45 GHz. Health Physics Journal, Vol. 21, Dec. 1971.

McRee, D. I.; and Walsh, P. J.: Microwave exposure system for biological specimens. The Review of Scientific Instruments, Vol. 42, Number 12, Dec. 1971.

McRee, D. I.; and Pendergrass F. T.: Interaction of a 2450 MHz microwave field with thermocouples and thermistors. Health Physics Journal. (In Press)

Serial No.: NIEHS-RSB-006
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Ultraviolet Dosimetry"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATOR: P. J. Walsh, Ph.D.

COOPERATING UNIT: Chemical Mutagenesis Branch

MAN YEARS

Total:	0
Professional:	0
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to measure the actual dose to the cells when exposed to ultraviolet radiation and to develop analytical dose response relationships. A comparison of the relative biological effectiveness of the ultraviolet radiation with ionizing radiation will be studied.

METHODS EMPLOYED: An ultraviolet exposure unit using 2537 Angstrom wavelength source is being designed. The unit will be used to produce damage in cell cultures. A UV photomultiplier tube will be used to determine the amount of energy absorbed by the cells.

MAJOR FINDINGS: An ultraviolet source has been obtained and calibrated. An exposure chamber which allows a variation in dose rate has been built. Seventy-seven percent of the energy output of the source is at 2537 Angstrom wavelength. The power density of the source ranges from 2 ergs/sec-mm^2 at 40 inches to 90 ergs/sec-mm^2 at 6 inches. In order to use the pulse height analyzer to measure the energy absorbed by the cells, the quantum density of the source must be drastically reduced. Spherical integrators have been designed, but have not as yet been built. These spherical integrators will reduce the quantum density and maintain the spectral integrity of the source.

Drs. Clive and Flamm of the Chemical Mutagenesis Branch have used the exposure system in an attempt to produce mutations. Dr. Phil Hamrick has used the system in an attempt to produce mutations in various strains of bacteria. No further

work on this project has been done during FY 73 due to the demands of projects in the microwave effects area.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information on the amount of ultraviolet energy absorbed by a cell to a given amount of damage is important in studying the repair mechanisms and in comparing this damage to that caused by other types of radiation or agents. Knowledge of the actual dose to the cells can lead to analytical descriptions of the interaction which may be applied to other types of cell damage.

PROPOSED COURSE: The spherical integrators will be incorporated into the UV readout system so that not only the exposure to the cells but also the dose to or absorption of the cells can be measured using the photomultiplier-pulse height analyzer system.

The dose of ultraviolet radiation to cell suspensions and cultures will be measured, and theoretical dosimetric concepts which describe the phenomena will be made. A comparison of the relative biological effectiveness of the ultraviolet radiation to ionizing radiation will be performed in an attempt to develop broad dose-response relationships.

Serial No.: NIEHS-RSB-008
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Variable Frequency Microwave Exposure System"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATOR: R. H. Wyatt, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.20
Professional:	0.20
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to design, build and calibrate a variable frequency exposure system for biological material.

METHODS EMPLOYED: Specifications were written for the desired system. A field uniformity at the specimen of 90 percent over a 4-inch diameter circle, a continuous frequency variation in the range of 1 to 10 GHz and a power level range at the specimen of 0.010 to 10 mW/cm^2 were specified.

MAJOR FINDINGS: Many of the biological effects of microwave radiation are frequency dependent. In terms of whole body irradiation, a frequency of 150-1,200 MHz affects the internal organs, whereas, a frequency of 10,000 MHz and above affects primarily the skin. Components of cells such as RNA, DNA and protein were found to have maximum absorption at different frequencies. Therefore, it is necessary to have the capability of varying frequency when investigating microwave effects on biological systems. The frequency range of 1 to 10 GHz was chosen because it is within this range of frequencies to which the general population is exposed.

All components for the system have been obtained and assembled. It was necessary to order 4 three-port circulators and a triple stub turner to protect the TWT-amplifiers and obtain maximum power output. These components were not in the original design, but have now been obtained and incorporated into the system. National Bureau of Standards has loaned a probe which is calibrated between 1

and 4 GHz. With this probe, the field patterns at the specimen were 90% uniform over a 4-inch diameter circle. Power levels of 40 mW/cm^2 and higher were obtained through this frequency range.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many biological effects of microwave radiation appear to be frequency specific. The variable frequency exposure system will provide the researchers of the Institute the capability of studying the effects of different frequencies on biological systems.

PROPOSED COURSE: A broad-band field intensity probe which will be calibrated from 1-10 GHz has been ordered from NBS. The fields will be calibrated from 1-10 GHz when this probe arrives. Also pin modulators which will enable the study of the effects of modulated and pulsed radiation will be ordered and incorporated into the system.

Serial No.: NIEHS-RSB-009
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Development of a Middle Ear Transducer"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Reginald O. Cook

OTHER INVESTIGATORS: L. Royster, Ph.D., NCSU; W. G. Thomas, Ph.D., UNC

COOPERATING UNITS: Audiology Research Laboratory, University of N. C. Medical School, Chapel Hill, N. C.

MAN YEARS

Total:	.7
Professional:	.4
Other:	.3

PROJECT DESCRIPTION

OBJECTIVES: The objective was to develop a mechanical, direct contact transducer which would be used to deliver very precisely known signals to the ossicular chain of experimental animals.

METHODS EMPLOYED: Because of its simplicity, small size and low power requirement, a parallel type piezoelectric transducer was selected for use. After many prototype models had been built (circa 50) several models which had the correct characteristics were evolved. Considerable data was taken from guinea pig ears during a two-week session at UNC's auditory research laboratory.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a device for delivering precisely quantified signals to the inner ear is a significant step forward, because it is so very difficult to accomplish this with acoustical (air borne) signals. One cannot very well say what the ear has done with acoustical information if he does not know precisely what the signal was at the eardrum.

Serial No.: NIEHS-RSB-010
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Adaptation of Zwicker loudness calculation scheme for annoyance evaluation"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. O. Cook

OTHER INVESTIGATORS: F. D. Hart, Ph.D., Director, Center for Acoustic Studies North Carolina State University; S. E. Dunn, Ph.D., North Carolina State Unive sity

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is the redesign of the Zwicker Nomographs for calculations which measure the annoyance rather than the loudness of sounds.

METHODS EMPLOYED: Mathematical and graphical techniques, along with the curves for equal annoyance developed by K. D. Kryter, will be employed. Results will be compared with results of judgment tests previously published in the literature.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The method developed by Zwicker mimics the sound energy summation properties of the human auditory system by correctly accounting for spread of masking and critical band properties.

It was developed, however, for subjective loudness calculations, which seem to be the preferred subjective parameter in Europe, whereas the annoyance parameter has been more widely used in this country. The methods developed by Kryter for annoyance estimation give good results as long as the spectrum is a relatively broad band, but are subject to error where energy peaks are widely separated in the spectrum.

PROPOSED COURSE: To redesign the Zwicker Nomographs for calculations which measure the annoyance of sounds. Nothing has been done on this during this reporting period. May make some progress before reporting period is up.

Serial No.: NIEHS-RSB-011
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Determination of Impedance of Middle Ear at two or three Atmospheres Pressure"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: J. Farmer, M.D., Duke University; G. Thomas, Ph.D., University of North Carolina at Chapel Hill, N. C.; Reginald O. Cook, Nat'l. Institute of Environmental Health Sciences

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS:

Total:	0.1
Professional:	0.1
Other:	0.0 (except for volunteer subjects)

PROJECT DESCRIPTION

OBJECTIVES: The objectives are to gain information relative to the change of impedance with increased pressure.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At present, only sketchy data exists on the sound transmissibility of the ear under several atmospheres pressure. Knowledge of transmissibility characteristics will make possible a better understanding of the permanent and temporary threshold shifts noted in personnel using the Duke University hyper-baric chamber.

PROPOSED COURSE: Data was taken from three subjects. Our intention is to gather more data at greater pressures before publication. More data was taken, but we still do not have enough to publish yet. This project should be terminated this year.

Serial No.: NIEHS-RSB-012
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of the Hearing Loss Threshold Curve for Young vs. Mature Animals" (Amended from previous year's title)

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: Reginald O. Cook and Stephen A. Falk, M.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.3
Professional:	0.8
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this project is to discover whether young guinea pigs suffer more morphological damage than more mature animals when exposed to identical high level noise conditions.

METHODS USED: A High Level, Broad Band Noise (110-120 dB; 20-20,000 Hz) environment has been created and maintained in the sound room while the animals were exposed for a period of 1 week. Damage is being assessed by whole body autopsy, and phase contrast microscopy of the cochlea, which is nearly complete, as of this writing.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The subject research should help shed light on the question: are infants' or children's hearing more easily damaged by environmental noise insult than adults'--a group from which all hearing damage data has come. The research has obvious practical implications.

Serial No.: NIEHS-RSB-013
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Ototoxicity of Methyl Mercury"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: R. Klein, M.D., Joseph Haseman, Ph.D., Gary Sanders, Fred Talley

COOPERATING UNITS: Pathology, Biostatistics, Animal Science & Technology

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to identify the pathological effects of methyl mercury on the cochlea by electronmicroscopic studies as a sequel to our previous studies.

METHODS EMPLOYED: Young Hartley guinea pigs were treated with daily subcutaneous doses of 2 mg/Hg in the form of methyl mercury hydroxide per kg body weight five times per week for 1, 2, 4, and 6 weeks. Hearing was assessed by the Preyer reflex, and the cochlea were examined by transmission electronmicroscopy with special study of the stria vascularis, spiral ganglion and auditory nerve.

MAJOR FINDINGS: The treated animals gained weight less rapidly than did the controls. After 3 weeks' treatment, they exhibited decreased activity but did not manifest any of the clinical neurological signs of methyl mercury poisoning such as ataxia, weakness and hind-limb crossing as seen in rats after similar treatment. Guinea pigs are more resistant than the rat to the toxic effects of organic mercurials.

After careful and thorough electronmicroscopic evaluation of the stria vascularis, including chromophil, chromophobe and basal cells and capillaries, spiral ganglion and auditory nerve, no consistent pathological changes of even a slight degree could be detected.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In our previous studies of methyl mercury ototoxicity, we found by the use of cochleographic reconstructions which record cell for cell pathology of surface preparations of the organ of Corti that the outer hair cells (OHC) were significantly damaged ($P<.05$) at the level of 2 1/2 turns from the cochlear base. There was a suggestion of pathological changes occurring at 3 1/2, 1 1/2 and 1/2 turns from the cochlear base, but such changes were not significant. Thus, from these studies, we have learned that methyl mercury hydroxide exerts an ototoxic effect on the OHC directly. Cochlear lesions from methyl mercury are very unique. All the known ototoxic drugs (aminoglycoside antibiotics, potent diuretics) and noise cause cochlear pathology initially in the first turn from the base. As dosage is increased or as treatment time is lengthened, pathological changes are observed progressively at more apical turns of the cochlea. Thus, methyl mercury represents a unique ototoxic agent, and it is hoped it can be used as a tool for revealing mechanisms of ototoxicity in general. Also these studies have elucidated the reason for the sensorineural hearing loss which has been observed in Minamata disease. Such a hearing loss is secondary to a primary cochlear lesion and to pathology of temporal lobe of cerebral cortex.

PROPOSED COURSE: This concludes our work in studying the ototoxic effects of methyl mercury.

PUBLICATION

Falk, S. A., Haseman, J., Klein, R. and Falk, M. K.: Methyl mercury ototoxicity: A model for the surface preparation technique, submitted to The Laryngoscope.

Falk, S. A., Klein, R., Sanders, G., Talley, F., and Lim, D. J.: The ototoxicity of methyl mercury, to be submitted to The Laryngoscope.

Serial No.: NIEHS-RSB-014
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "The Combined Ototoxicity of Sodium Salicylate and Ethacrynic Acid"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: Mrs. Terry Frye, Dr. J. Prazma, Ann Groce

COOPERATING UNITS: Division of Otolaryngology, University of North Carolina School of Medicine.

MAN YEARS

Total:	1.5
Professional:	1.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Salicylates, including aspirin, and ethacrynic acid, are well recognized as having ototoxic effects on the cochlea. The objectives of this project are to determine their effects when administered simultaneously.

We previously demonstrated potentiation of ototoxic effects of sodium salicylate and ethacrynic acid by electrophysiological means. Further pathological studies were done with electronmicroscopy of organ of Corti and stria vascularis and light microscopy.

METHODS EMPLOYED: Groups of young Hartley guinea pigs were teated with Na salicylate (100 mg/kg) subcutaneously and ethacrynic acid (5 mg/kg) ip. daily both simultaneously and separately for 4 weeks, and pathological studies were performed by transmission electronmicroscopy of organ of Corti and stria vascularis and by phase contrast microscopy of surface preparations of organ of Corti.

MAJOR FINDINGS: We found a low level (3-4%) of outer hair cell degeneration in the cochlea not localized to any turns in animals treated with ethacrynic acid, and similar results were found for Na salicylate. Compared to controls, the damage was statistically significant at a level just acceptable ($P = .05$). In animals treated with both drugs, 6% damage was observed. We were thus un-

able to confirm our previous impression of potentiation of damage from the agents, only additive effect occurred.

By electronmicroscopy, we found edema of outer hair cells rather consistently in Na salicylate treated animals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Salicylates have been regarded as causing a completely reversible hearing loss. Our findings indicate that a permanent loss of extremely small degree (hair cell degeneration is irreversible) may also occur with salicylates. Clinically, this may be undetectable by present audiometric techniques when considering that there is a redundancy of the hair cell population. Some can be lost in low frequency regions of the cochlea without an elevation in hearing threshold.

Except for vasoconstriction, no consistent pathological change in inner ear has been demonstrated from salicylates by either light or electronmicroscopic studies. It is unknown how aspirin causes hearing loss. These studies localize aspirin effect to outer hair cell pathology.

PROPOSED COURSE: We will do further studies of a same nature to settle the matter of whether a low level permanent damage results from salicylates and whether our initial electronmicroscopic findings are reproducible.

Serial No.: NIEHS-RSB-015
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Incubator Noise and Possible Deafness"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: J. Farmer, M.D.

COOPERATING UNITS: Division of Otolaryngology, Duke University

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives are to answer two questions--1) What noise levels are present in functioning pediatric incubators? and 2) Are such noise levels damaging, or potentially damaging, to the hearing mechanism?

METHODS EMPLOYED: A sound level meter with octave band analyzer was used to measure sound pressure levels in functioning infant incubators. The following sound measurements were made for each incubator and for the background noise: A-weighted sound level, unweighted (linear) sound level, and an octave band frequency analysis with center frequencies of the bands being 31.5, 63, 125, 500, 1000, 2000 and 4000 Hz.

MAJOR FINDINGS: The frequency distribution of the incubator noise followed a consistent pattern among incubators with peak levels between 31.5 Hz and 250 Hz. Above 250 Hz, there was a rapid drop off in sound pressure level so that 99% of the sound energy was distributed below 500 Hz. The average A-weighted and unweighted sound pressure levels are 57.7 dB and 74.5 dB respectively.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: According to present damage-risk criteria for exposure to noise, the above sound levels would not be considered a risk to hearing. However, considering that present damage-risk criteria are based on studies using adult humans and not newborns, that the infant in the incubator is exposed to continuous noise and not intermittent as was used in establishing the present criteria, that con-

tinuous noise is known to be more ototoxic than intermittent, and that infants are often exposed to ototoxic antibiotics, which are known to potentiate the ototoxic effects of noise, such noise levels as were found can be considered as possibly damaging to the inner ear.

PROPOSED COURSE: The project as described in last year's project report was written up and will be shortly published in The Archives of Otolaryngology.

This report concludes this project number.

PUBLICATION

Falk, S. A. and Farmer, J., Incubator noise and possible deafness, Archives of Otolaryngology. (In press)

Serial No.: NIEHS-RSB-016
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of 2450 MHz Microwave Radiation on Biological Material"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: P. E. Hamrick, Ph.D.

OTHER INVESTIGATORS: D. I. McRee, Ph.D. and P. J. Walsh, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	0.5
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects.

METHODS EMPLOYED: Several strains of bacteria, one bacteriophage, and solutions of DNA have been exposed to 2450 MHz microwaves at power levels sufficient to maintain a temperature of 37°C. Bacterial growth rate curves and bacteriophage lysis time have been compared in exposed and control situations. Thermal denaturation curves of control and exposed DNA solutions have been compared with the aid of an ultraviolet spectrophotometer equipped with a temperature programmer.

MAJOR FINDINGS: Studies have been made which suggest that "non-thermal" microwave fields (fields which do not significantly increase the temperature of the specimen) at various frequencies interact with intracellular components to alter metabolic and/or genetic processes. Interpretation of these effects is difficult because mechanisms are not specified and in many cases the exposure parameters are not known quantitatively. Many of the effects appear to be very frequency dependent. For example, the growth rate of Escherichia coli has been shown to be enhanced or retarded depending on the frequency in the range 65-75 GHz. When Escherichia coli and Pseudomonas aeruginosa were exposed to 2450 MHz microwaves at 60 mW/cm^2 and held at 37°C, no effects on

growth rate were observed. Under similar conditions no effect was observed on the lysis time of bacteriophage T-2 or on the thermal denaturation curves of DNA. Thus far, our studies indicate that if there are "non-thermal" effects at 2450 MHz then these effects are relatively small in the systems we have investigated. Growth rate, phage production and lysis time are systems very sensitive to disturbing influences because the interaction of many cellular components is involved. The sensitivity of the DNA solutions to thermal effects was varied by adjusting the ionic strength of the solutions. However, no sensitivity to microwaves has been detected at 2450 MHz and with exposure levels up to 200 mW/cm^2.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to determine if "non-thermal" effects are present, their magnitude with respect to thermal and if there is any interaction between thermal and "non-thermal" effects. Thermal effects on biological materials have been extensively studied; consequently, it is very important to evaluate the role of "non-thermal" effects. If these are minor, then hazard analysis can be pursued on the basis of known results of subjection of biological specimens to temperature increases.

Serial No.: NIEHS-RSB-017
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Determination of Trace Elements in Biological Samples"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATORS: P. J. Walsh, Ph.D. and P. Hamrick, Ph.D.

OTHER INVESTIGATORS: N. Underwood, Ph.D., University of North Carolina

COOPERATING UNITS: Department of Environmental Sciences and Engineering, University of North Carolina

MAN YEARS

Total:	0.8
Professional:	0.6
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: This project was initiated to develop quantitative techniques for the determination of trace amounts of elements, singly and in combination, in biological samples.

METHODS EMPLOYED: X-ray emission spectrometry is a useful technique for the determination of elements in biological samples. It has the advantage of speed of analysis because of its ability to analyze many elements simultaneously and its high detection efficiency. In recent years, the combination of semiconductor detectors with field-effect-transistor input preamplifiers and the optimization of that combination has yielded the resolution necessary to allow separation of the characteristic X-rays for all elements above atomic number 12.

MAJOR FINDINGS: A system is in hand with demonstrated sensitivity in the parts per million range. A new system has been designed and is being fabricated offering substantial improvements over the present system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aside from its value as a conventional analytical tool, X-ray emission spectrometry offers the possibility of in vivo studies in animals and man ranging from the study of the distribution of iodine in thyroid glands to measurement of deposition of aerosols in the respiratory tract. A variety of studies normally

carried out by the use of radioactive tracers appear within the capabilities of X-ray emission spectrometry.

PROPOSED COURSE: We plan to continue the development of X-ray emission spectrometry as an analytical tool and, in conjunction with NIEHS-RSB-001 and the Lovelace Foundation contract, to explore its application to the determination of aerosol deposition in models and *in vivo*.

Serial No.: NIEHS-RSB-018
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "A Cross-Sectional Area Measuring Device for Whole Biological Samples"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. H. Wyatt, Ph.D.

OTHER INVESTIGATORS: D. A. Dahl

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is the design and construction of an electromechanical instrument for measuring the cross-sectional areas of a whole, unsectioned biological specimen (or any small object of irregular shape) as a function of the specimen length. Moreover, the feasibility and the usefulness of this device are to be investigated.

METHODS EMPLOYED: The theory of the operation of the device is based on a fundamental principle of fluid mechanics: any body immersed in a liquid is acted on by an upward buoyant force equal to the weight of the displaced liquid. Using this principle, the pertinent equations are derived in the preceding annual report on this project.

In a system built, electromechanical techniques are employed for sensing and processing the continuous weight signal as the object is slowly lowered into the fluid filled vessel. A mechanical, motor-driven apparatus is used for lowering the object; digital micrologic circuitry allows much of the motor control to be performed automatically. To date, water is the only fluid to be used in the immersion vessel. A linear, unbonded, strain gage transducing cell senses the weight signal. The four elements of the cell form a fully active, purely resistive, wheatstone bridge circuit, operating on direct current. The transducer signal is then fed to a modular, differential amplifier employing differential input circuitry with high common mode noise rejection and extremely high

input impedance. The output signal voltage is developed single-ended with respect to system common. It is then differentiated by a circuit utilizing operational amplifiers with compensation for capacitor leakage incorporated. The signal from the differentiator is filtered by a special 5.0 Hz low-pass filter; this filter has constant amplitude response and a linear phase characteristic for negligible signal distortion. The signal from the filter is then put on a wide display, strip-chart recorder where system calibration is performed. The present design of the instrument will yield a device for measuring area cross-sections on objects weighing up to 50 or 60 gms. and having a gross shape-volume characteristic of up to something like a small apple.

MAJOR FINDINGS: The work to now has shown the major problem areas to be: 1) the inherent mechanical vibrations of the motor drive being picked up by the weight sensor, and 2) surface tension effects causing artifact at the entrance (or exit) of the object into the fluid. There are several versions of electro-microbalances available. These offer a sensitive weighing device with precision mechanical and electrical components which perhaps could be modified and used in the continuous weighing system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This instrument should enable our local scientists to obtain length versus area characteristics on whole, unsectioned, biological samples of various types (e.g., fetuses, tumors, etc.), at a large savings in man-hours. A possible application of the instrument is in the study of biological objects with abnormally unsymmetrical morphology, e.g., achondroplasia, micromelia, and swellings of various types.

PROPOSED COURSE: Concerning the problem of reducing motor drive vibration noise transmitted to the weight sensor, a new and inherently quieter, hysteresis, multispeed motor has been obtained and is operable at 5 different syncronous speeds. A new lead screw of 80 threads per inch has been prepared and will lower the frequency bandwidth containing measurement information. This should allow more effective noise filtering.

In conjunction with the preceding, an interesting new method to electronically sense and cancel out the perturbing mechanical noise is to be developed and tried. This noise cancellation technique depends on being able to sense independently the mechanical noise signal and then subtracting this from the signal containing weight information with additive noise.

An alternative method to investigate is the use of the already refined precision weighing mechanism of available electrobalances. Because the weighing mechanism of these balances is electrodynamic, depending on the interaction of 2 magnetic fields, it is reasonable to expect this mechanism to be less susceptible to mechanical noise disturbances. Also the required differentiation circuit could be added. Here the biological object would remain fixed to the weighing device, and the fluid vessel would be raised for immersing the biological sample.

Various methods of dealing with the surface tension effects are to be considered, e.g., pre-wetting, use of immersing fluids with low surface tension characteristic, etc. However, because this effect produces predominate artifact only at

the beginning and end of a measurement result, it is perhaps feasible that the identifiable artifact could just be ignored. However, there is some limit as to how sharp or discrete an area variation can be accurately detected.

Serial No.: NIEHS-RSB-019
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of Non-Thermal Microwave Radiation Effects on Neurological Functioning"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: R. H. Wyatt, Ph.D.

OTHER INVESTIGATORS: D. I. McRee, Ph.D.

COOPERATING UNITS: Duke University, Dept. of Electrical Engineering, Dept. of Physiology

MAN YEARS

Total:	0.75
Professional:	0.75
Other:	0.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to conduct an investigation into the non-thermal effects of microwave radiation on neurological functioning.

METHODS EMPLOYED: The presently accepted working definition of a non-thermal effect is a biological change attributable to irradiation by microwave energy without significant change of body temperature. This definition does not exclude the possibility of specific local heating effects occurring even though there is no change of the overall or average temperature of the organism. To give a possible example for means of illustration: Conceivably, electric current flow in the cerebrospinal fluid could cause localized heating of the central nervous tissue. A change of temperature in the area of the hypothalamus amounting to not more than 0.1°C could provoke a generalized response of the antonomic nervous system. Such a response would be classed non-thermal inasmuch as it would not be a direct physical consequence of the energy input delivered by the irradiation.

The real question is: Does a non-thermal effect of low level radiation occur? If yes, is it harmful? The effects of microwave radiation can, in principle, be studied at four levels.

1) Whole Organisms

From the point of view of environmental health, important questions are: Is there a change in the behavior, metabolism, circulation, and other functions of men and animals exposed to microwave energy? Is there an immediate effect during short-term exposure? Is there a delayed effect during prolonged exposure? Is there an after effect following exposure?

2) Organs and Cells within Whole Organisms

The first thoughts here are toward mammalian nervous system effects. Subjects of additional studies may be the circulatory and endocrine organs.

3) Isolated Organs and Isolated Cells

With respect to this level, we currently have a contract with Duke University for the study of microwave-neuron interactions.

4) Model (synthetic) Systems, such as artificial membranes.

Present concentration should be directed at the first two levels of study, and in this respect neural effects in cats and rats will be investigated. The surviving spinal cord of functionally "decapitated" cats provides a convenient test system for a variety of elementary functions of the mammalian nervous system. By stimulation and recording of neural discharges in selected nerves and spinal roots, one can test synaptic transmission, reflex facilitation, potentiation (as a measure of transmitter mobilization), and various forms of synaptic inhibition.

Electrical recording with microprobes enables the observation of individual nerve cells, their membrane potential, and synaptic functions. Such observations in a mammalian organism would complement those made in isolated ganglia of cold blooded animals. It is important to recognize that from the behavior of the latter that of the former cannot always be predicted. While the spinal cord offers many advantages for the study of elementary neural functions, attention should be paid also to other parts of the nervous system. Particularly, a report of evoked potential waves in brain stem structures, using no other stimulation than pulsed microwaves, requires careful reproduction.

In all studies in which metal probes are used, great care will have to be taken in guarding against artifacts. Electrodes and cables will have to be checked in "dummy" runs for induced currents, rectification, and heat production. The evoked potentials mentioned above remain suspect until proven true by careful experiments.

MAJOR FINDINGS: A review of the literature and a study of the research requirements are essentially complete. Because methods and results of many reported experiments are questionable and vague, it would be of value to duplicate to some degree certain ones reporting results that, if valid, are of significance. Concerning behavorial effects in rats, there is a report suggesting that rats exposed to microwave radiation for days or weeks show reduced levels of activity. For the tests performed, the common factor in all observations was the overall slowing of animal activity. Unfortunately, the magnitude of the depression was not given. A different, independent investigator applied thermographic techniques in checking the accuracy of the field intensity reported for

the experiment; he found that in certain areas of the exposure chamber, it was of the order of 10 times greater than the value reported. If behavorial effects are shown to occur, one would then ask questions on the mechanism. Presently, we do not have facilities for behavorial studies related to microwave effects; hopefully in the near future we will obtain such. Also, as mentioned in the preceding section, another experiment that should be reproduced carefully is that of microwave induced evoked potentials in the brain stem. The planned neurophysiological experiments in mammals, where the organs and cells are not removed from their own environment, will be complemented by the present Duke University contract work on isolated cells and organs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The hazards of microwave radiation are a priority matter of current concern for NIEHS. The study of biological effects caused by microwave radiation forms the basis for determining safe levels of exposure. Presently, the safe level of continuous exposure in the U.S.A. is 10 mW/cm^2. This is based on one underlying assumption: namely, that the possible damage due to microwave radiation exposure is of a thermal nature. However, many other experiments, especially from Soviet-bloc nations, report indications of nonthermal, neurological effects like headaches, fatigue, hyperactivity, altered brain wave patterns, and changes in the functional state of nerves, e.g., induced transmembrane potentials. These observations reportedly occurred at radiation levels below the extant U.S.A. safe levels. It is to be recognized that the safe exposure level in the U.S. is 1000 times greater than that set for Soviet nations. The question of the nonthermal influence of microwaves is the principal one in the problems and consequences of the biological effects of microwaves; it must be resolved before the degree of the hazard posed to man is determined.

PROPOSED COURSE: Specific factors in the design of experiments to study neural effects in cats and rats are being pursued. This includes consideration of modifications to the existing microwave system that might be necessary, as well as a study of the equipment needed in neurophysiological experiments in mammals. Also, another aspect to be considered is the possible effect of microwaves on the enzyme acetylcholine esterase, which catalyzes the breakdown of acetylcholine, a common interneural transmitter chemical.

Serial No.: NIEHS-RSB-020
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Services Branch

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Effects of Microwave Radiation on the Embryonic Development of Japanese Quail"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: D. I. McRee, Ph.D., P. E. Hamrick, Ph.D.

OTHER INVESTIGATOR: None

COOPERATING UNITS: Poultry Science Dept., N. C. State University (Consultants)

MAN YEARS

Total:	0.6
Professional:	0.4
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to determine the effects of microwave radiation on differentiating cells systems.

METHODS EMPLOYED: Fertilized Japanese quail eggs will be exposed to microwave radiation in the frequency range of 1-10 GHz. The power level will initially be set to maintain a temperature of between 34° and 37°C. in the quail egg. At these temperatures any effects due to the microwave radiation exposure should be nonthermal. If effects on cell differentiation is noted at these power density levels, the power level will be decreased until a threshold value is determined. The initial end point for observation of harmful effects will be deformities in the fully developed embryo.

MAJOR FINDINGS: Reported data in the literature on the exposure of fertilized chicken eggs to microwave radiation has shown gross deformities. Due to the power density levels and the temperature of the eggs during exposure, there is controversy over whether the effects were thermal or nonthermal. In our experiments, we intend to determine if the effects are nonthermal in nature. Investigation into the power density level necessary to maintain a temperature of 35°C. ±1.5°C. in the quail eggs have been determined. Both an infrared microscope and liquid crystals have been used to measure the egg temperature. The power density level has been determined to be approximately 60 mW/cm^2. It

has been observed that the energy absorption of the egg depends on the size and shape parameters. This requires the selection of eggs which are as close to the same size and shape as possible. A 20 egg holder has been built for holding the eggs during exposure, and the variation in temperature in the 20 eggs varied by ±1.5°C.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evaluation of whether microwave radiation affects differentiating cell systems is a basic question which must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

PROPOSED COURSE: The study of where and if hot spots occur in the quail egg, even though the average temperature is at the desired value, will be performed. Quail eggs will then be exposed to a level such that no portion of the egg is above the level where thermal effects occur. The eggs will be placed in an incubator after exposure until the eggs hatch. Deformations in the hatched quail will be observed and compared with control eggs.

Serial No.: NIEHS-RSB-021
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Measurement of Energy Absorption from Microwave Fields"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: P. J. Walsh, Ph.D.

OTHER INVESTIGATORS: Daryl Mercer, Graduate Student, UNC

COOPERATING UNITS: Department of Environmental Sciences and Engineering, University of North Carolina

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Measurement of the absorption of microwave energy on the basis of fundamental physical laws.

METHODS EMPLOYED: Microwaves are absorbed in a spherical shell of dielectric material enclosing a dry gas. Temperature changes of the dielectric are transmitted to the gas which expands in accord with the ideal gas law over the range of pressures and temperatures encountered.

MAJOR FINDINGS: A double-walled spherical glass bulb in current use with saline dielectric and dry air gas exhibits sensitivities which will allow measurement at field levels comparable to current U.S. exposure limits.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Measurement of dose or absorbed energy is generally more desirable than measurement of field intensity. A device which allows measurement of absorbed energy as well as exhibits adherence to simple physical laws has been a primary goal in radiation dosimetry.

PROPOSED COURSE: The response of the system will be determined over a frequency range of 1-10 GHz. Its utility for determining the absorption properties of various materials will be investigated.

Serial No.: NIEHS-RSB-022
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Noise in the Hospital"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: Nancy F. Woods, RN, MN

COOPERATING UNITS: Dept. of Nursing
Duke University Medical Center
Durham, N. C.

MAN YEARS

Total:	.5
Professional:	.5
Other:	.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are to identify the sources and quantify the levels of excessive noise present in the hospital and to relate these levels to pathophysiological effects on the hospitalized patient.

METHODS EMPLOYED: A sound level meter with octave band analyzer was used to measure sound pressure levels in functioning infant incubators and in a 17 bed surgical recovery room and a 7 bed acute care unit (for post-operative care of cardiac surgical patients) consisting of room 1 and 2 at Duke Hospital. The predominant sources of the noise-equipment, personnel, procedures, etc.--were noted. To provide a complete evaluation of noise levels over 24 hours, measurements were made for 10 seconds at 5 minute intervals and were correlated with the number of hospital staff and patients and with other sources.

MAJOR FINDINGS: The average A-weighted and unweighted sound pressure levels of the incubators are 57.7 dB and 74.5 dB respectively with a frequency distribution between 31.5 and 250 Hz. The levels of noise generated by individual mechanical equipment, personnel, and patients averaged 65.6 dB(A) and 80.0 dB (linear) and emanated from multiple sources including suction machines, respirators, cardiac monitors, oxygen outlets, bedpan washer, moving beds, X-raying patient, deliverying supplies, child crying, and suctioning patients. Some noise sources were necessary, others were not. The 24 hour noise surveys

revealed that the average noise level in the recovery room is higher during the day than night with an average of 59 dB(A) for 7:00 a.m. to 3:00 p.m., 58 dB(A) for 3:00 p.m. to 11:00 p.m. and 55 dB(A) for 11:00 p.m. to 7:00 a.m. The range is 45-84 dB(A). The range in room 1 and 2 of the acute care unit is 50-70 dB(A) and 50-76 dB(A).

In all locations, noise level is highly correlated with number of staff, and in the recovery room, noise is also associated with number of patients. If attention is restricted to periods having at least one patient, the average noise levels are elevated to 60.1 dB(A) and 73.3 dB (linear) for room 1 and 55.8 dB(A) and 68.1 dB (linear) for room 2.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These noise levels were evaluated for adverse effects on the hospitalized patient by careful consideration of our present knowledge of the physiological effects of noise on the pituitary-adrenal axis and the cardiovascular and auditory systems.

Like other stressful stimuli, such as trauma, surgery, infection, and burns, noise can be considered a nonspecific stress which releases ACTH from the pituitary. A level of noise as low as 68 dB (linear) (which is less than the average noise present in the recovery room, acute care unit, and incubators in this study) for only 30 minutes releases ACTH as measured by a decrease in adrenal ascorbic acid content and by cosinopenia, a peripheral glucocorticoid effect. In humans, a 65 dB sound at 10,000 Hz causes a 53% increase in plasma 17-hydroxycorticosteroids. There is indirect evidence that noise-induced adrenal changes are transient, disappearing with cessation of the noise. However, there is no evidence that the hypothalamo-hypophyseal-adrenal axis adapts to the stress of chronically maintained noise.

Although data in humans is incomplete, it is highly probable that noise levels as measured in this study stimulate the hypophyseal-adrenocortical axis of hospitalized patients. Because these patients are already in stressful situations from multiple causes including surgery, infection, hemorrhage, and fear, it would appear wise to decrease any further stress to which they are exposed. Although only an approximate criterion, average noise levels should therefore be kept below 65 dB (linear).

Our evaluations of the adverse cardiovascular effects of hospital noise is still in progress.

Based on present criteria of 80 dB(A) for the production of hearing loss, the hospital noise levels measured in thisstudy are not a danger to hearing. However, these criteria are not applicable to the hospitalized patient because they are based on intermittent 8 hour per day exposures, not continuous ones, which are present in the hospital and which are more damaging than intermittent which provides an opportunity for recovery. Also they are based on studies in adults and do not strictly apply to infants. Also recent studies demonstrating potentiation of the damaging effects of noise and ototoxic aminoglycosidic antibiotics can be applied to hospitalized patients. A much larger amount of hair cell damage in the organ of Corti occurs in guinea pigs upon exposure to kanamycin and noise levels of 58 dB(A) compared to exposure to

either agent alone. The amount of damage greatly exceeds that expected from adding the separate effects of each agent. A similar level of noise is also present in the incubators, recovery room and acute care unit in this study.

Recommendations for decreasing noise levels were arrived at. A lowering of noise levels can be achieved by improved acoustical design of existing facilities and better acoustical consultation in erecting new facilities. Also physicians and nursing personnel can lessen unnecessary noise levels.

Serial No.: NIEHS-RSB-023
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Investigation of the Combined Insult of Noise and Salicylates on Morphological Damage to the Ear"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Reginald O. Cook and Stephen A. Falk, M.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.3
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To ascertain whether salicylates potentiate damage from noise insult.

METHODS EMPLOYED: A high level precisely defined noise environment was set up in the sound room and 3 week old guinea pigs exposed to 120 dB for 96 hours continuously while receiving daily doses of 100 mg/Kg salicylates subcutaneously. Cochlear damage will be assessed by phase contrast microscopy.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possibility of synergism between these two most common ototoxic agents has wide range human population implications. Salicylates in high doses are known to cause temporary hearing shifts, but this is thought to be nearly fully recoverable where no noise is involved.

Serial No.: NIEHS-RSB-024
1. Research Services Branch
2. Biophysics & Instrumentation Section
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Determination of the Movement of the Ossicular Chain in Response to Sound"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Reginald O. Cook

OTHER INVESTIGATORS: None

COOPERATING UNITS: Audiology Research Laboratory, University of N. C. Medical School

MAN YEARS

Total:	.5
Professional:	.3
Other:	.2

PROJECT DESCRIPTION

OBJECTIVES: A new, optic, non-contact, displacement measuring device was adapted to measure the output of the piezoelectric device described in project NIEHS-RSB-009, subsequently it was found that this device could be used to measure the displacement of the umbo, the termination of the ossicular chain in the center of the tympanic membrane, in response to sound. Purpose was to ascertain displacement vs. sound pressure level.

METHODS EMPLOYED: The frequency and level of sound at the level of the tympanic membrane were measured simultaneously with the displacement of the umbo. Set up consisted of probe tube microphone, micromanipulators, a 40 μ gram, 30 mill reflector on the umbo, optic sensor, and careful positioning in a vibration free environment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We have demonstrated capability to measure small deflections (3-5 A) across frequency (0-20 KHz) without use of an expensive laser inferometer. The technique we have domonstrated, may be adaptable for cochlea input measurements in aural physiology.

OFFICE OF THE ASSOCIATE DIRECTOR FOR SCIENTIFIC INFORMATION AND COMMUNICATION

OFFICE OF THE ASSOCIATE DIRECTOR FOR
SCIENTIFIC INFORMATION AND COMMUNICATION
Summary Statement

Scientific Liaison with Categorical Environmental Programs

The survey of Federal programs dealing with environmental health research made last year for the OST-CEQ Ad Hoc Committee established a network of personal communication that has stood the Institute in good stead this year. In some instances copies of project sheets and other program documents are now sent automatically by activities that hardly communicated at all before. In most other cases a telephone call is all that is necessary to get information. Periodic visits serve to keep our files reasonably current. Time and the activities of a previous staff member have helped to break down an earlier suspicion that NIEHS had predatory intentions. NIEHS is consulted more frequently by other activities on matters of mutual interest. Lack of spontaneity is noticeable with only one major program. Direct scientist-to-scientist communication has been greatly increased.

Quick Retrieval System for Scientific Information

The principal target for this activity is now the project information received from other programs. A fairly quick mode of access, although still manual, has been developed. The system can be readily automated if the conduct of a survey is authorized by higher echelons. A Project Board is maintained giving reference numbers to documents dealing with elements of other programs.

Intramural Scientific Information Service (including Library)

This continues to expand, but the Library, and consequently other functions, is badly handicapped by lack of space. Miscellaneous documents, which are often the source of needed information, are no more than roughly classified owing to lack of shelf space. A move to new quarters will be very welcome. A MEDLINE terminal has been installed and is now operating. As its usefulness gets appreciation, the work load can be expected to increase.

Scientific Information Evaluation and Reporting Service

This activity is now taking form as a multi-level mode of promulgating considered opinion and authoritative review of complex environmental problems on which only isolated and partial statements were previously available. The four books compiled for non-specialists in conjunction with the Fogarty International Center seem to be reaching a fairly wide audience. A very favorable response has been received from the readership of Environmental Health Perspectives after only two numbers. (The third number is just appearing.)

Review articles sent to the periodical Environmental Research are in good demand. NIEHS seems to be developing a reputation for being the source of well-balanced statements on several tricky problems. Participation in conferences organized by the New York Academy of Sciences has served to brighten this image. Critical comments on draft action documents sent for review by such programs as EPA and NIOSH have earned respect, even when they have not evoked much happiness. Although the intent is peripheral to its mission, NIEHS is now fairly frequently asked to participate, and to review the evidence, at sessions in which industry, labor, medicine, and science jointly consider some of the more difficult environmental problems.

Beryllium Case Registry

Support for this activity will be assumed by NIOSH when the present term expires in May.

International Programs

The responsibility of this Office is limited to operation of the bilateral agreement with France relative to pulmonary effects of environmental contaminants. Arrangements have been made for exchange of personnel in FY73 and FY74. We participated in the XVII International Congress on Occupational Health in Buenos Aires and will be involved with the AAAS conference in Mexico City on Man and Environment.

Editorial Committee

The number of papers submitted for clearance over the period July 1 to April 1 was 103.

Serial No.: NIEHS-OSI-001
1. Office of Associate Director for Scientific Information & Communication
2. Liaison
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Scientific Liaison with Categorical Environmental Health Programs"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: Categorical Environmental Health Programs

MAN YEARS

Total:	1.1
Professional:	0.3
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: The primary objective is to maintain awareness of relevant scientific and technical activities between the intramural operations of NIEHS and other Federal environmental programs. Formal processes support, but do not satisfy ultimate objectives of liaison -- meeting together, thinking together, working together.

BACKGROUND: One of the reasons advanced for creating NIEHS was the necessity for a facility which would provide the more basic, longer-term, multiple factor research necessary to the development of criteria, standards, and control measures by the categorical environmental health programs. This necessitates continuing exchange of information on problems, needed research, investigative activities, and emergent scientific information between the research and categorical activities. The need was expressed in announcements by the Surgeon General on the implementation of NIEHS, and has been the subject of frequent inquiry by OMB and DHEW representatives.

MAJOR ACCOMPLISHMENTS: The ready flow of project information from agencies across the Federal Government concerned with environmental health has continued. The currency of files, including relevant legislation, is checked at periodic visits to the agencies. Pending clarification of a continued survey under the auspices of higher echelons, the information has not been coded or indexed in detail. Retrieval is possible only by manual search.

PROPOSED COURSE: Continued acquisition of project material from relevant programs combined with updating at personal visits.

Serial No.: NIEHS-OSI-002
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Quick Retrieval System for Scientific Information"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Richard K. West

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	0.7
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The objective is the development and operation of a system for quick retrieval of information on scientific topics relevant to NIEHS programs.

BACKGROUND: The relatively unstructured nature of information obtained from categorical programs (see Project OSI-001) and other sources imposes the need for some system of retrieving information on a particular subject with speed and precision. The preparation of position and other staff papers (see Project OSI-005) also demands quick access to important relevant information. Intramural scientists need assistance in securing references, abstracts, and original documents relating to topics of immediate interest. It is desirable that one system be established for handling information acquired to meet these internal needs.

MAJOR ACCOMPLISHMENTS: The system has been modified to concentrate on project information received from other programs and to be capable of responding to any directive from higher echelons for repeated review of Federal programs.

PROPOSED COURSE: Develop system for automatic search and compilation of project sheets as directed by higher echelons.

Serial No.: NIEHS-OSI-003
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "NEHSC Library"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Ralph Hester

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.8
Professional:	0.3
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: To provide necessary library holdings and services for the use of NEHSC personnel.

BACKGROUND: Although the three neighboring universities provide excellent backup library resources, and the services of both the NIH library and the National Library of Medicine are available to NIEHS, a moderate library facility is essential in the immediate vicinity to minimize the time spent by scientists in travel and absence from active work, and to facilitate consultation of relevant material.

MAJOR ACCOMPLISHMENTS: Space in present quarters in Building 10 is saturated. Plans for organization of more space have been prepared. There are now 218 periodicals regularly received. Demands for interlibrary loans average 385/month. The annual purchase budget is $35,000.

PROPOSED COURSE: Move to larger space in Building 10 when available. Increased coverage as new Branches are established in the Institute.

Serial No.: NIEHS-OSI-004
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Intramural Scientific Information Service"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Ralph Hester

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.7
Professional	0.7
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is to provide relevant scientific information by both routine and demand services to NEHSC personnel.

BACKGROUND: Both the preparation of position and other staff papers, and the consideration of relevant scientific literature by working intramural scientists, requires the acquisition, assembly, and referencing of documentary information from a wide range of sources. Both the Scientific Directorate and the intramural scientists require assistance in (a) using the Quick Retrieval System (Project NIEHS-OSI-002); and (b) going beyond the limited internal library resources (Project NIEHS-OSI-003) to external sources.

MAJOR ACCOMPLISHMENTS: Information Services now constitutes a Section in the functional organization of OSIC, charged with securing such scientific information as is required by Institute members for the various purposes. This includes normal library operations (Project NIEHS-OSI-003), personal services according to profiled requirements, and meeting demands for special purposes. It provides an essential input to the growing demand for position papers on a variety of subjects (Project NIEHS-OSI-005). Much of the information secured becomes potential input to the QRS (Project NIEHS-OSI-002). A MEDLINE terminal has been installed. It will share a line with UNC and Duke University Medical School libraries.

PROPOSED COURSE: Increased services with access to additional external sources.

Serial No.: NIEHS-OSI-005
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Scientific Information Evaluation and Reporting Service"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.9
Professional:	0.7
Other:	1.2

PROJECT DESCRIPTION

OBJECTIVES: The objective is to evaluate the relative significance of various environmental stresses and preparation of reports on selected topics in environmental health.

BACKGROUND: a. Efficient allocation of Institute resources to specific investigations presupposes a knowledge of the range of environmental situations that affect man, the relative seriousness of their effects, the feasibility of ameliorating the results, and the extent of investigative attention already being given to the significant situations.

b. The Directorate is frequently called upon to present position papers on various environmental health matters of national import.

MAJOR ACCOMPLISHMENTS: The fourth book prepared in conjunction with the Fogarty International Center and the proceedings of the NYAS Conference on Coal Workers' Pneumoconiosis have been published. The NYAS Conference on Pulmonary Reactions to Organic Substances was held in November. Three numbers of Environmental Health Perspectives have been issued. Material for two more numbers has been assembled. Authorization is being sought to continue this as a permanent publication. The work with the Ad Hoc Committee on Environmental Health Research is under review by higher echelons. The Ecology Committee has almost completed its task. Organization has started on a Handbook on Environmental Physiology for the American Physiological Society.

PROPOSED COURSE: Continue with Environmental Health Perspectives, and organization of the Handbook on Environmental Physiology. Work with the University of Florida Medical School to produce publications on environmental medicine.

Serial No.: NIEHS-OSI-009
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Participation in International Programs"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The objective is to participate in those aspects of international programs operating through DHEW that are relevant to the NIEHS mission.

BACKGROUND: With the transfer of the categorical environmental programs out of DHEW, NIEHS becomes a mainstay of DHEW interests in environmental health. This applied particularly to international programs, in which environmental aspects are getting increasing attention. Close cooperation is maintained in this connection with the Fogarty International Center and with the Office of International Health.

MAJOR ACCOMPLISHMENTS: Under the bilateral agreement with France, Dr. J. Knelson (EPA) visited France for 4 weeks as the appointee for FY73, and arrangements are being made for Dr. Pham to come to the USA for 4-6 weeks in April-May. Dr. B. Ferris (Harvard) will be the visitor to France for FY74, and probably Dr. Voisin will come from France.

Dr. Lee participated in the XVII Congress on Occupational Health in Buenos Aires, Argentina, in September 1972.

A Russian delegation visited NIEHS for a week under the bilateral arrangement with DHEW. Several other foreign visitors have been received, including a group of 7 from the Japanese Scientific Research Council.

PROPOSED COURSE: Continue bilateral arrangements with France. Participate in a AAAS Conference in Mexico City in July 1973. Cooperate with other developments as necessary.

Serial No.: NIEHS-OSI-010
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

PROJECT TITLE: "Special Studies"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is to organize and conduct special studies as required.

BACKGROUND: From time to time special studies not fitting into other portions of the organization are needed, and devolve on members of the Director's staff.

MAJOR ACCOMPLISHMENTS: Drinking water from domestic catchment cisterns in St. Thomas, Virgin Islands, was collected, and older houses were examined for peeling lead-based paint. Roadside soil samples were collected. Analyses are being made.

PROPOSED COURSE: None at present.

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

INSTITUTE DIRECTOR'S SUMMARY STATEMENT ON EXTRAMURAL PROGRAMS

The Extramural Program continues to be an effective means of involving highly qualified scientists on a national basis in environmental health research. During the year the National Institute of Environmental Health Sciences grantees have contributed in an important way toward identification of new and solution of previously-recognized environmental problems. Concomitantly, they have made significant advances in understanding the underlying biochemical, physiological, and pathological mechanisms of environmental factors in carcinogenesis, teratogenesis, mutagenesis, neural and behavioral disorders, and in the evolution of certain pulmonary and cardiovascular diseases. University-based Centers and Program Projects continue to serve as national resources for long-term multifaceted research on problems of high national priority and for rapid response to new problems in their respective areas of expertise. On occasion, Center and Program Directors have taken the initiative in organizing national and international conferences to provide a forum for exchange of information in specific areas of environmental health science and related fields and for discussion of new findings with important social and industrial implications. During the coming year the Institute will establish a new Center with special competence and unusual scope in epidemiology of environmental diseases, including, but not limited to, those caused by asbestos.

Fiscal restraints in FY-1973 have made necessary a further effort to bring grant-supported projects into closer alignment with the Institute mandate. Toward this end, several competing renewal projects were replaced by new grants with outstanding scientific merit and program relevance. In the coming year support for individual research grants will diminish to one-third of the total Extramural budget, resulting in approximately equal distribution of funds among individual projects, program projects and University-based Centers. Reduction in support for individual grants reflects only the tractability of this area of the program and not an insensitivity to the value of individual project grants. Indeed the necessity to reduce support is clearly unfortunate both because of the shrinkage in vitality of the research effort and because of the loss to the overall program of the intrinsic value of individual grants. Project grants bring maximum scope and diversity of talent to the program; they act as safeguards against program biases that sometimes develop in directed and team research; and, in addition to their contributions toward solution of universal problems, they often afford close proximity to special hazards involving enclaves of risk populations at the occupational and community levels.

Curtailment of new grants has a further, more subtle effect on mission oriented programs as the number of grants becomes critically small. It is recognized that selection and funding of grants in accordance with priority scores has a salutory effect on scientific sophistication. However, there is an inherent bias in the peer review system in favor of proposals based upon prior research and understanding. This is amplified in new fields of research where highly sophisticated bases have not yet been formed. The smaller the number of applications that can be funded, the greater the

tendancy for research to become concentrated in areas not necessarily consonant with program needs. Because of the variety of problems and the paucity of prior research in some program areas, this problem has begun to surface in environmental health science. Thus ways are currently being explored to maintain balance in the Institute's extramural programs, and, at the same time, to preserve the cherished originality and excellence of submitted applications.

The necessary steps have been taken for orderly phaseout of Research Training Programs. A predictable consequence of this action is intensification of the shortage of trained manpower in environmental health science and an increasing lag time in critical evaluation of some 1000 new food additives, industrial chemicals, pesticides and other potentially hazardous substances coming annually into the chemical milieu to which man is exposed. It is expected that some increase in environmental health scientists will occur gradually through entrance of students now involved in ongoing research programs into the field. However, because an effective means of rectifying the manpower deficit is not now visible, use of extramural programs for maximum near-term, as well as long-term, national impact has become imperative.

SUMMARY STATEMENT
OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

RESEARCH GRANTS

The number of research grant applications assigned to the National Institute of Environmental Health Sciences in fiscal year 1973 declined 17 percent from the 1972 figure. The proportion of approvals remained essentially the same; however, only 17 new research grants were funded, a decrease of 53 percent from the number funded in 1972. This reflects both a decrease of 4.5 percent in allocated funds and an increase of 15 percent in the number of noncompeting projects funded in 1972. The number of competing renewals and supplements declined 16 and 87 percent, respectively. On balance, the number of research grants (129) remained essentially the same as last year (128) with total funding of $8.574 million as compared with $8.966 million a year ago.

TRAINING PROGRAMS

The official Plan for Phaseout of the National Institutes of Health Research Training Programs was stated in a memorandum dated February 9, 1973 from the Acting Director, National Institutes of Health. All research training programs are affected by the policy, including regular training grants, fellowships, traineeships, Research Career Development Awards, and all special awards. Staff has already taken the necessary steps to comply with all aspects of the policy and will continue to act in accordance with the National Institutes of Health guidelines on a case-by-case basis. Under the phaseout plan no new training programs will be started unless a firm commitment was made prior to January 29, 1973. Applications for which initial review was not completed prior to that date have been administratively withdrawn. During the phaseout period budgets of training grants will be adjusted downward as the number of trainees decreases. Research Career Awards will be honored for the duration of the career provided the awardee continues to meet the standards described in the January 1, 1963 policy statement for the program.

During FY-1973, the National Institute of Environmental Health Sciences made commitments for support of 29 regular competing and noncompeting training programs prior to the effective date of the phaseout. These involved 246 students, a reduction of 23 percent in the number supported the previous year. Total funds were reduced to $2.5 million, 19 percent below the FY-1972 level. Both the number of trainees and support funds will decrease further during the next fiscal year as students complete their training and current commitments are fulfilled. The number of fellowships decreased from 20 last year to 11 prior to phaseout. A commitment had been made for one new Research Career Development Award, which will be honored in accord with the National Institutes of Health guidelines.

The impact of the phaseout of environmental health sciences training is expected to be substantial on environmental health programs in government, industry, universities and, ultimately, on the public at large. As pointed out in the "Narrative and Justification for the National Institute of Environmental Health Sciences FY-1974 Training Program Budget Request", career

development in the environmental health sciences requires a new type of interdisciplinary training. It must be problem-oriented and must supplement and broaden the training received in traditional biomedical disciplines. It is readily apparent that present needs and demands for trained personnel far exceed the supply. Many program directors in the scientific community estimate that the national demand at the present time exceeds the supply by a factor of three. Moreover, it is estimated that there is a lead-time of four to six years in the preparation of a Ph.D. graduate for independent research. At present only a dedicated few students are likely to undertake the necessary additional training at their own expense to begin careers as competent environmental scientists. It seems clear, therefore, that phaseout of training programs will have a more adverse impact on progress toward national goals in environmental health science than in the more traditional disciplines. Manpower needs will have to be filled through on-the-job orientation and experience instead of pre- or postdoctoral training in the university. This will increase substantially the lead-time from the Ph.D. degree to highly productive research. Ultimately it can be expected to slow the progress of research and to increase the time interval between identification of environmental problems and development of adequate information for appropriate responses by regulatory agencies and the Congress.

UNIVERSITY-BASED CENTERS

University-based Centers generally evolve through program grants. Their forte is the unique ability to perform multifaceted, high priority research in environmental health science and to provide laboratory and other training across traditional disciplinary lines for highly qualified young people who intend to pursue careers in environmental health science or in closely related fields. Obviously, the challenges in environmental health science are so numerous and varied that no one group can hope to achieve excellence in all areas of concern. Thus, the strategy in serving the national need optimally with available funds is to provide support for several Centers, each with special staff expertise in one or more areas of relevance to the mission of the institute. During FY-1973, the National Institute of Environmental Health Sciences supported six Centers with funds totalling $3.55 million.

Effective communication between the Centers and the Institute was maintained through the Environmental Health Sciences Advisory Council's Subcommittee for University-based Centers. Four meetings of the Subcommittee with Center staffs and students were held during the past year. Dr. Edward M. Cohart, Department of Epidemiology and Public Health, Yale University, served as chairman of the Subcommittee. Details of his reports on the meetings are recorded in the minutes of the meetings of the National Advisory Environmental Health Sciences Council. In brief, the Subcommittee considered the Centers to be excellent national resources, with the exception of the Washington University Center. Because of the predominant orientation of the latter toward ecological problems instead of health, the majority of the programs were considered peripheral to the mission of the National Institute of Environmental Health Sciences. Therefore, phaseout of the Center was recommended and approved by the National Advisory Environmental Health Sciences Council and is now is progress. All support will be discontinued after this fiscal year.

Recommendations for creation of a new Center at Mount Sinai School of Medicine, New York City, under the direction of Dr. Irving Selikoff, was presented to the National Advisory Environmental Health Sciences Council for consideration at the March, 1973 meeting. As approved, this Center will provide the Institute a strong program in epidemiology in a metropolitan area ideally suited for studies of a number of important environmental problems. Particular emphasis will be placed on diseases and predisposition to diseases related to inhalation of asbestos particles.

Biannual meetings of Center Directors were convened on May 7-8, 1972, at the National Institute of Environmental Health Sciences facility in North Carolina, on October 26-28, 1972, at the Center for Study of the Human Environment, University of Cincinnati, Cincinnati, Ohio, and May 20-23, 1973, at Oregon State University, Corvallis, Oregon. The purpose of the Center Directors' Meetings is to provide a forum for exchange of information and discussion of mutual concerns among the Directors, the National Advisory Environmental Health Sciences Council Center Subcommittee, the National Institute of Environmental Health Sciences Director and his staff and invited officials and scientists of sister agencies with interfacing interests in environmental health science.

SUPPORTED RESEARCH ACCOMPLISHMENTS

Important advances were made by grantees during the past year toward development of understanding of the interrelationships between physical and chemical factors in the environment and human diseases. The program in every sense was a national effort and each type of grant contributed in its special way toward remarkably coherent cumulative results. Highlights of a number of grants which are illustrative, but by no means all-inclusive, of the scope and accomplishments of the program are presented herewith.

RESEARCH HIGHLIGHTS

Through the Extramural Program, the Institute supports a national research effort toward development of an understanding of the interrelationships between hazardous chemical and physical factors in the environment and human diseases. It acts also as a catalyst in the translation of the knowledge developed through grant-supported research into planned action to diminish or relieve health problems of environmental origin. The following highlights from selected individual, program, and University-based Center grants are illustrative, but not all inclusive, of the scope and accomplishments of the Program during the past year.

NEW DISEASES AND ETIOLOGICAL RELATIONSHIPS

Interrelationships between environmental factors and important diseases of man are already clearly established. Some familiar examples are emphysema, chronic bronchitis, pneumoconiosis, farmer's lung, byssinosis, and some types of cancer, hypertension, birth defects and mental disorders. Moreover, there is increasing evidence of subtle physiological and heritable anomalies traceable to environmental factors. Research by grantees during the past year has provided further insight into the magnitude of health hazards of environmental origin through discovery of a number of new diseases and etiological relationships.

New Cancer-inducing Agents. Grantees at the Institute of Environmental Medicine, New York University has shown that polyester "fiberglass" and polyurethane particle suspensions, generated under conditions simulating occupational inhalation exposures, produce emphysema and cancer of the lung lining in rats. Further studies on the haloethers, previously shown to be potent skin carcinogens, have established that the compounds will induce carcinomas of the lung and nasal linings as well as of the skin. These findings have had important ramifications in government agencies and in industry. One previous manufacturer has stopped production of the haloethers and others have taken steps to minimize exposure of employees to them. The National Institute for Occupational Safety and Health (NIOSH) undertook studies to evaluate the effects on humans, because the original study was conducted on rodents. Presumptive evidence of increased carcinomas in humans has since been found, confirming the potential hazard of the material to exposed workers. To define the problem in terms of quantitative dosage responses, NIOSH provided additional funds for further studies on humans. At the same time, experiments were undertaken to develop safety criteria and standards on which to base realistic control measures and for use by the Department of Labor in surveillance and control actions with respect to manufacturers. Concomitantly, the National Cancer Institute (NCI) placed this class of compounds in its registry of known carcinogens and has sponsored further research on the relationship between molecular structure and carcinogenicity of the haloethers and to explore more intensively the possible carcinogenicity of other important industrial chemicals. NIEHS continues to provide basic support to determine the mechanism by which the compounds induce cancer in order to develop a rationale for prediction of the carcinogenicity of chemicals

and the levels and circumstances of exposure that can be expected to lead to cancer.

New naturally-occurring tumor-inducing agents have been found by a grantee at Purdue University. These are by-products of three species of Penicillium. When fed to mice with their regular diets, the molds induced tumors in the lining and spongy tissues around the bronchioles and alveoli of the lung. Though the toxins have not yet been characterized chemically, they appear to be different from any known toxins since the nature of the tumors and pathology is different from that produced by known purified Penicillium toxins.

Cocarcinogens and Tumor Promoters. A number of studies of basic aspects of cocarcinogenesis and tumor promotion by noncarcinogenic agents are supported by NIEHS. In addition to the well-known promoting agents croton oil and tobacco tars, grantees have shown that certain fatty alcohols also show weak promoting activity. Others at Oregon State University have found that cyclopropenoid fatty acids are powerful promoting or cocarcinogenic agents for aflatoxins in liver cancer development in rainbow trout. These substances are natural components of some food and oil products, such as cottonseed oil, kapok oil, okra and wheat, which also serve as excellent substrates for the mold which produces aflatoxins as well as other toxin-producing molds.

Lung Cancer Enhancers. Considerable evidence is accumulating that development of lung cancer is often enhanced by air pollutants which are not carcinogenic under the usual circumstances of exposure. This appears to be especially true in the case of sulfur dioxide, as shown by grantees at the New York University Institute of Environmental Health. Early results from tests with nitrogen oxides also indicate an increase in metaplasias in rats exposed to combinations of the gases and proximal carcinogens such as benzpyrene and methylcholanthrene. Moreover, tests in the same laboratory show that mixtures of the two proximal carcinogens cause a greater incidence of lung cancer than expected from the equivalent amounts of the compounds administered alone. These studies suggest that the risk of lung cancer is likely to be increased in situations where mixtures of toxic agents prevail and that tests of individual carcinogens alone do not provide an adequate index of population risks.

Pentachlorophenol. While cancer is one of the more visible environmental-related diseases, several other potential problems of a different nature have been brought into view by NIEHS grantees. A study being conducted in Hawaii indicates that there are significant levels of pentachlorophenol (PCP) in all human urine and blood samples taken at random in the state. Whether or not this constitutes a general health problem has never been considered. PCP is a somewhat volatile compound used in large quantities as a preservative for wood and pilings, as a molluscicide, fungicide, herbicide, pre-harvest desiccant, and in processing some plastic material which often comes into contact with food products. Despite the presumption, based on experiments with animals, that PCP is relatively innocuous, there have been a significant number of intoxications among occupationally exposed individuals in recent years, some of them fatal. The few analytical data available for blood and

urine from fatal poisonings indicated that the lethal amount in these fluids exceeds the amounts found in occupationally exposed individuals by as little as a factor of two. This would appear to be a very narrow margin of safety. Moreover, it was shown that the compound is not excreted by humans as readily as by animals and that there is a sex difference in susceptibility in rats, with the male being considerably more susceptible. The long-term effects of PCP are unknown but these findings suggest a potential new problem deserving attention, expecially in view of the fact that PCP's may contain dioxins as contaminants.

Phthalate Esters. Phthalate esters (PE) are used in the plastics industry as plasticizers. Usage for all purposes is estimated at a billion pounds annually. Reports from several laboratories during the past year indicate ubiquitous distribution of these compounds in the environment. Concentrations in fish and other aquatic organisms is tenfold greater than DDT, PCB, and other chlorinated hydrocarbons combined. Fortunately, the toxicity of PE's is very low and their interest to the National Institute of Environmental Health Sciences stems primarily from the work of grantees at the Johns Hopkins University. Their original work established the fact that there is significant migration of PE's from plastic blood packs into human blood used for transfusion. Direct injection or infusion of toxicants into the bloodstream generally constitutes a greater potential risk than other modes of entry into the body. It was found that the esters tend to accumulate in the lungs and that there are some effects on blood platelet function at very high levels. Attempts are being made currently to determine if other subtle toxic effects occur on long-term exposure and repeated transfusion. These findings may necessitate development of new methods of whole blood and plasma storage in order to assure a supply of unadulterated blood even if present levels of PE's in the environment prove to be a little consequence to wildlife and the general population.

Mycotoxins. New disease syndromes associated with intake of moldy foods were brought to light by three different laboratories under NIEHS grants. It was demonstrated that some species of Penicillium fed along with a normal diet to mice produced strong phototoxic responses characterized by erythema and edema and necrosis of the ears, muzzle, paws, and tails of the mice upon subsequent exposure of the animals to light. Keratitis and blindness occurred in some cases. Other Penicillium species were found which caused degeneration of hepatocytes and degenerative changes and malfunction of the liver and kidney. Workers at Vanderbilt University have implicated metabolites of Penicillium spp. in a neurological anomaly of sheep, cattle and horses in Tennessee. The compounds are commonly referred to as tremorigens because of the quivering and loss of muscular control characteristic of early symptoms of poisoning. These symptoms are usually followed in a matter of hours by death in severe cases.

Phytotoxins. The Vanderbilt University workers have found substances in sweet potato infected with Fusarium solani (javanicum) which cause respiratory distress, usually followed by death, in cattle ingesting infected potatoes. Post mortem findings are primarily an interstitial type of pneumonia with severe pulmonary emphysema and edema. The principal edemagenic substance has been isolated and given the name 4-ipomeanol.

Sulfur dioxide, Ozone, and Oxides of Nitrogen have been presumptively implicated in systemic effects by several grantees. It was previously considered that these substances produced local effects in the linings of the airways of the lung and were of little further concern. However, it has been shown that sulfur dioxide may react with sulfur-containing components of the lung lining to form S-sulfonates. These may then be absorbed into the bloodstream and become distributed to all parts of the body where the complexes break down to regenerate sulfur dioxide. Work with ozone, which generally makes up about 90 percent of the oxidant level of the atmosphere, suggests that ozone may also interact at the lung surface with unsaturated fats to form ozonides and peroxides. Like ozone, these are also quite toxic and may be distributed by the bloodstream to other parts of the body. With this hypothesis, the possible effects of ozonides and peroxides on fat metabolizing systems are being studied with special reference to effects on lipoprotein structure and stability and implications of these effects in development of atheroslerosis and other circulatory diseases.

Polychlorinated Biphenyls. The hazard of polychlorinated biphenyl compounds (PCB's) was realized several years ago as a result of accidental poisoning of humans and animals. Because the compounds are not highly toxic, public concern is much like that associated with the DDT problem. An NIEHS grantee at Cornell University has worked for a number of years on the effects of chlorinated hydrocarbons and recently extended his studies to the PCB's. His purpose is to determine the multigeneration effects of the compounds at levels which produce no overt toxicity. Using the dove as a model, he has shown that PCB at levels that have no effect on the first generation causes heavy embryonic mortality and skeletal deformations in the second. Mortality and deformation are associated with chromosome breakage in cells of the allantoic sac and in limb buds of the embryo. Whether similar embryonic damage occurs in mammals or humans remains to be determined, but birds have historically been important harbingers of risk associated with the chlorinated hydrocarbons. This work adds a new dimension to the concern about this group of compounds and points to the importance of multi-generation considerations in assessing risk associated with environmental agents.

Lead. A new aspect of lead intoxication pertinent particularly to children comes from investigators at the University of North Carolina. The research indicates a close relationship between nutrition and the behavioral effects of lead. Deficiencies of calcium, iron, and certain other minerals increase the toxicity of lead by a factor of 20. Further, it was shown that growth and maturation of the nervous system is significantly retarded because of a retardation of the rate of myelination. These effects parallel those seen in chronic plumbism in children and suggest that a combination of malnutrition and lead intake will enhance the mental retardation associated with chronic lead poisoning. In psychometric tests, it was shown that anomalies in the performance of rats parallel the retardation of myelination in the nervous system. These findings contribute to identification of basic effects of environmental lead on individuals in the general population who are likely to be at greatest risk at a given lead level.

Zinc and Copper. A diet low in essential metals may make one more vulnerable to heart attack. Conversely, manipulation of certain metals in the diet may

offer a way to control dangerous levels of fats in the blood. These hypotheses derive from researchers at the University of Cincinnati, supported in part by a Center Grant from NIEHS. The scientists have found - apparently for the first time - a relationship between blood fat levels and the intake of zinc and copper. They found that when the body content of zinc and copper is raised, blood fat levels decrease. Conversely, when the metals decrease, fat levels rise. Although the studies are limited to rats at the present time, they may have important implications for man, because serum fat levels have been related to atheroslerosis and coronary heart disease in man. This finding should add a new dimension to both the experimental and clinical studies of the role of lipids in human cardiovascular diseases. From the standpoint of environmental health, it raises questions regarding copper and zinc interactions with agents which counter their effects or bind them strongly to create deficiencies at the metabolic level sufficient to cause elevation blood fat levels.

TOXICANT CONVERSIONS

An important activity closely allied with identification of new hazards and etiological relations is identification of new toxicants formed from man-made and natural toxins by living organisms and physical factors in the environment. NIEHS supports several projects with this primary goal. Illustrative of these are projects at Iowa State University, the University of Texas, Jefferson Medical College, and the University of Illinois.

Work at Iowa State University and in numerous other laboratories has shown that organophosphorothioate insecticides are susceptible to biological oxidation. The products of conversion may be many times more toxic than the original compound. The best known and most important example of this is the conversion of the insecticide parathion to paraoxon, although related insecticides in this class also undergo the reaction.

Work on the fate of aromatic hydrocarbons in the environment has led to a growing recognition of the importance of oxidative conversions in petroleum components. Volatile petroleum products are important components of polluted air over urban areas and there is evidence that potentially carcinogenic aromatic constituents of oil become much more potent carcinogens upon oxidation. Somewhat related studies deal with the common and important industrial solvent benzene. Benzene readily penetrates the skin and attacks the red blood cells, but there is evidence that it must first be metabolized before the typical benzene effect is produced. This is indicated by the fact that if metabolism is blocked with metyrapone, no effect on red blood cells is evident. The goal of studies at Jefferson Medical College is to determine the toxic by-products of benzene metabolism. At the present time only one product, e.g., phenol, has been identified in the mammalian body. However, based on theoretical chemical considerations, an unstable, highly reactive epoxide should be formed and this is postulated as the active agent in red blood cell destruction. Attempts are now being made to prove the identity of the intermediate and further clarify its mode of action.

It has been shown by a number of scientists that upon exposure to sunlight the insecticides aldrin and dieldrin are converted to derivatives called

photoaldrin and photodieldrin. These derivatives are more toxic than the original compounds, but it is postulated that there is a further conversion in the mammalian body which enhances toxicity because the photoderivatives per se do not exhibit maximum toxicity if tested in such a way as to prevent further conversion. A grantee at the University of Illinois is attempting to clarify the chemistry of the next step in the conversion and has evidence that the photoderivatives are metabolized to ketones, which may be the ultimate toxicants.

Fundamental studies of the kinds mentioned are considered essential in assessing the health hazard and understanding the nature of the hazard associated with these compounds in the environment. In its simplest terms, the work is concerned with identifying the precise nature of primary toxicants.

PHYSICAL FACTORS

With the growing complexities of technology has come recognition of the influence of a number of physical environmental factors on human well-being. Among the more important are radiation in various forms, noise, microwaves, and visual pollution. NIEHS supports research in several of these areas.

Laser Radiation is a relatively new field of investigation if physics and biology. Its use as an experimental tool is growing rapidly and it has some significant potential therapeutic applications. Little is known about the consequences of long-term exposure of long-range effects of short exposures; therefore, NIEHS supports a grant at Northwestern University for work by Dr. Karl Weiss on multiphoton excitation. This has potential significance with respect to health hazards associated with electromagnetic radiation. Though the short-term objectives of this research is generation of fundamental knowledge of a relatively new source of energy, it may well have possible practical application in guarding against the consequences of laser radiation as more and more people become exposed to it.

Environmental Lighting and Metabolism. This is also an area in which relatively little is known in terms of consequences to human health. However, a considerable amount of information is available which indicates varied effects of light on biological processes. For example, light is involved in photo-sensitization phenomena in certain skin diseases and allergies. It has more subtle effects on biological rhythms and regulatory systems such as ovulation, which, like vision, are mediated by photoreceptors that change the energy of light into nerve impulses. More recently it has been established that light, acting through the eyes and subsequently the brain and nervous system, affects the activities of the pineal gland, which is believed to be important in triggering and controlling some of the biological rhythms in human physiology. Dr. Richard Wurtman at Massachusetts Institute of Technology is attempting to learn more about the effects of environmental lighting and various components of light on these rhythmic processes and particularly on those under pineal gland control. He has found indications that the retinal pigment of the eye, rhodopsin, apparently is the receptor responsibile for signaling the pineal gland to activate the enzyme responsibile for the final step in formation of dark skin pigments.

Temperature and Dehydration. Studies by Daetjer and others at Johns Hopkins University are designed to determine the effects of dehydration and extreme temperatures on susceptibility to environmental agents. The studies show that dehydration following water deprivation of experimental animals results in a significant, progressive decrease in the number of macrophages in the lungs, suggesting that dehydration may make the lungs more susceptible to damage by particulate air pollutants. Exposure to high temperatures (95° F) increased the number of macrophages during a 24-hour period of exposure but the number decreased to normal after 48 hours. Exposure to cold (42° F) had no effect for 24 hours but caused a significant decrease in the macrophage count after 48 hours, suggesting that cold may result in impairment of lung defenses.

Other studies on human responses to the thermal environment (by Hardy and others at the John B. Pierce Foundation) have led to the development of three temperature indices of the thermal environment which provide key information in relation to man's responses to heat, cold and work. It was shown through quantitative studies on animals that there is a thermosensitive region in the posterior hypothalamus that sends neural signals which govern the animals conscious behavior without stimulating involuntary physiological responses such as shivering. Experiments with humans show that the psychological and physiological responses of man to his environment are related to his own thermal comfort and well-being and to his health. The magnitude of discomfort is directly proportional to the wettedness of the skin during sweating, defined as the ratio of observed evaporative heat loss to the evaporative power of the environment. The utlimate objective of this program is to define in quantitative terms the psychophysics of the senses of heat and cold and the relationship of these to man's functioning and well-being.

Biologic Effects of Radiation. Studies by Urbach at Temple University have led to development of lighting systems that closely simulate natural noon sunlight for irradiation of experimental animals. This equipment has permitted critical experiments in phototoxicity (effects of chemicals and light on the skin) and photocarcinogenicity. In addition, investigations on the genetics, anatomy and physiology of various hairless animals (mice, rats, pigs) have resulted in strains exceedingly useful for studies in phototoxicity and carcinogenicity.

A by-product of animal experimentation aimed at improvement of light treatment for hyperbilirubinemia of newborn infants, is the observation that the effects of increased bilirubin, the development of kernicterus (a brain change leading to permanent mental deficiency) can be arrested and to some extent reversed in experimental animals. The new light sources have also led to development of a reliable technique for determining phototoxic effects (sunburn augmentation) of a variety of chemicals in animals which is useful in screening for such effects in man. The possible side effects of intense illumination in connection with such studies and therapeutic treatments are now being investigated, including combined effects of light and oxygen on visual function and on circadian rhythms.

Using the new equipment, skin cancer can now be induced reliably in animal models. It has been shown that at least one compound, 8-methoxypsoralen, which is known to be phototoxic, is also photocarcinogenic. Other phototoxic compounds are being tested as photocarcinogenic enhancers. Studies are also underway on photochemical alterations in ozone and other chemicals in relation to skin cancer.

EPIDEMIOLOGY

Much has been accomplished in environmental health by use of animal models and short-term tests in humans. However, there are numerous problems in which time is a vital factor and for which we have no substitute for man as the test organism. Many of the problems are of such national and world-wide importance that they must be resolved without primary regard to cost through human population studies. At the present time the major efforts of Institute grantees center around asbestosis, cancer, pulmonary and heart diseases, and the long-term effects of lead and other environmental metals.

Asbestos Diseases. One of the more pressing epidemiological problems is asbestosis and asbestos-induced cancer, which is being investigated intensively by scientists at Mount Sinai School of Medicine in New York. Previous experience in this country and throughout the world with occupationally exposed asbestos workers leaves no doubt of the hazard of asbestos. It is ubiquitous; it is deadly on prolonged exposure; and it is perhaps the most insidious of all particulate air pollutants because its effects may not become clinically evident until 20, 30 or more years after exposure. Moreover, the no-effect level is still unknown. Theoretically, a single asbestos fiber can initiate a tumor. The current studies involve groups of people whose exposure to asbestos varies in degree and began at least 20 years ago. The rationale is to select populations which form an exposure gradient and to document the incidence of asbestosis and cancer over the lifetimes of the individuals. For example, one group consists of 23,000 men exposed to asbestos through their occupations in the building trades as roofers, tile setters, and foundation workers. These men worked on jobs in proximity to insulation workers, whose hazard has already been documented. A second group consists of families of asbestos workers. They were exposed through asbestos dusts brought home on the clothes and shoes of the workers and deposited in the home. A third group is made up of men who in 1942 lived 1/2 mile from an asbestos plant in an eastern city. Along with epidemiological data, quantitative data on tissue levels of asbestos particles will be collected through biopsy or autopsy when possible.

Bladder Cancer. During the past year, Dr. Phillip Cole at Harvard University has made available his results from population studies on bladder cancer among various occupational groups. The data show that bladder cancer in workers in the rubber and leather industries is as much as twice that in no-risk industries. Excess risk was also found among dyeworkers, painters, and certain organic chemists but the data were not statistically significant.

An unanticipated but potentially important finding was an apparent association between bladder cancer and coffee-drinking. The risk appeared somewhat greater for women than men. If the association is eventually shown to be casual,

one-fourth of bladder cancer in men and one-half that in women may be attributed to coffee-drinking. Further studies to verify this are in progress.

Radiation-induced Cancer. Dr. Roy Albert and his colleagues at New York University have made a second survey of about 2,000 children given X-ray treatment for tinea capitis (fungal infection of the scalp) between 1940 and 1958. The survey is about 75 percent complete. It reveals that the incidence of all cancers is 1.7 times higher in the irradiated group than in nonirradiated controls and the incidence of head and neck cancers is 3.8 times higher. A surprising finding is that the number of thyroid adenomas is four times normal in the irradiated children. This was unexpected because the thyroid received negligible exposure.

Air Pollution. A study underway since 1961 is continuing in Berlin, New Hampshire, by Drs. Benjamin Ferris and William Burgess of Harvard University to assess changes in the local environment with respect to smoking, respiratory symtoms and pulmonary function in a cross-section of the population. Results from a 1967 survey have now been compared with their 1961 survey. They conclude that there is a significant increase in respiratory problems and small changes in pulmonary function associated with the increases in air pollution in the area and there appeared to be a significant increase in cancer of the male genito-urinary system. The exact compositional changes in the air over Berlin were not reported, but the studies are indicative of increasing problems as the air pollution level considered *in toto* increases.

Carbon Monoxide. Carbon monoxide has been implicated as a causative agent in atherosclerosis and in aggravating coronary vascular disease. It is a commonly recognized by-product of cigarette smoking, but significant concentrations are also found in the air of large cities, in private homes and in steel plants. Thus, it is widely distributed and often unsuspected. Studies by Drs. Edward Radford and John Frazier at Johns Hopkins University seek to determine the relationship of carbon monoxide in the blood to mortality from arteriosclerotic coronary artery disease. They are also attempting to identify specific environmental sources of CO and to separate effects of CO inhaled during cigarette smoking from those attributable to CO from other sources. The criterion for measuring CO exposure is blood carboxyhemoglobin levels. Correlations are being made in several ways and some results are as follows. Records of the Medical Examiner's Office on patients with proved arterioslerotic heart disease show a correlation between increased carboxyhemoglobin in the blood and cigarette smoking. This is further correlated with a 5.8 percent increase in disease and mortality in the heavy smoking group. Correlations of CO measurements in private homes in the East Baltimore area show that 20 percent of the homes have detectable levels probably deriving from heating units. In four percent of the cases the concentration was above 20 ppm of air, which is considered a significant hazard over the long term.

The conclusion from these studies is that individuals from all walks of life who inhale smoke or breathe air in which combustion has occurred are, or may be, at risk, especially if the situation persists or if the individual is already under the stress of heavy smoking or preexisting heart disease.

Heavy Metals. Research initiated three years ago under Dr. David Suskind at the University of Cincinnati has shown a positive correlation between high atmospheric lead concentrations and high motor vehicle density. Comparison of recently obtained data with those obtained in 1961-62 demonstrates significant increases in lead in 14 of 19 sites sampled, with no decrease at any site. Blood samples from individuals in these areas show no consistent correlation between blood lead and atmospheric lead but in the larger metropolitan areas such as New York, Philadelphia and Chicago, the residents had consistently higher blood levels than suburban residents. Blood lead levels in smokers were significantly higher than in nonsmokers.

This group has also conducted a study of lead in hair as an index of lead exposure in Panama. They found the amount of lead in hair generally declines as the distance from Panama City and the Canal Zone increases. These results suggest that there is a rural to urban gradient in lead exposure around large cities.

Several other grantees are investigating the use of children's teeth as an index of cumulative lead exposure. Uptake and retention of lead in teeth is quite constant and the studies are expected to help close the gap in information about chronic, subclinical lead exposure in children, who, as a group, are more susceptible to lead intoxication than adults and are likely to suffer some form of neurological or mental effects over the long term if exposure is sufficiently high.

Sunlight and Skin Cancer. Extensive epidemiologic studies performed in Philadelphia, and with collaborators in Galway, Ireland and Queensland, Australia, have shown the importance of genetic as well as environmental factors in the induction of skin cancer in man. The Celtic type, with fair, freckled skin, light eyes, easy sunburning, and little tanning are the most predisposed individuals to skin cancer. An ultraviolet light television system has been developed by Dr. Frederick Urbach of Temple University which promises the possibility of easily and inexpensively identifying people who are particularly likely to develop skin cancer and other light-induced skin changes early in life. This is expected to lead to significant developments for prevention of effects and in counseling.

CARCINOGENESIS

Although studies on the basic aspects of carcinogenesis is the primary responsibility of the National Cancer Institute, significant work in this area relevant to environmental health is being carried out by scientists with NIEHS support at the New York University Institute of Environmental Medicine. Previous basic studies and follow-up studies during the past year indicate that transition from normal cells into cancerous cells involves several stages. Dr. Walter Troll, one of the project leaders, calls these the initiation stage, which appears to involve a mutation, and the promotion stage, in which the normal suppression of cell division no longer prevails. Initiating agents cause physical changes in DNA, modifying its ability to act as the template for RNA synthesis and intergrity. Initiation of tumors by certain compounds in the environment correlates with the ability of these

compounds to cause mutations in bacteria. Other substances, such as croton oil and other tumor-promoting agents, do not cause changes in DNA and do not induce mutations in bacteria. Instead, they have been found to activate protein digesting enzymes (proteases). The New York University scientists suggest that these enzymes cause mistranslation of genetic information to the rest of the cell so that the cells continue to divide.

In very recent studies, they find that compounds which inhibit the proteases also inhibit promotion of tumors by tumor-promoting agents. Thus, the second stage of cancer formation can be blocked. Other studies show that alkylating agents that react with DNA and have the inherent ability to cause tumor formation do not always give rise to tumors. This is taken as evidence that changes in DNA alone are not sufficient to cause cancer. In synthesizing all of the facts from their studies, the New York University group concludes that a metabolic and a genetic change are necessary for tumor formation and that the metabolic factor is a product of protein degradation or partial degradation. The product is still unidentified.

MUTAGENESIS

There is ample evidence from numerous sources that many environmental agents are mutagenic at sufficiently high doses. The primary problems now center around the questions of how much of a given mutagenic agent is actually required to induce mutations in man, and how these agents can be measured at realistic environmental levels so that the risk to man, if any, is reflected in a valid way. No satisfactory solution to either of these problems is at hand. Because of the obvious importance of mutagenesis in this and future generations, NIEHS supports several studies designed to assess various test systems for applicability to man and to develop a clearer understanding of the mutagenic process and factors that contribute to differences between organisms in their responses to specific agents.

Test Procedures. Dr. Raymond Harbison at Vanderbilt University is attempting to determine the usefulness of current mutagenesis testing procedures in animals and to develop additional capabilities for reliable testing. He is evaluating the dominant lethal assay for applicability to environmental monitoring, using known mutagenic agents. This test has been used widely in the past and Dr. Harbison finds that it is useful for detection of large mutations that result in death of the fetus. However, it provides no measure of less drastic mutations in which the animals survive with heritable changes in metabolism or minor anatomical defects.

Dr. William Lee at Louisiana State University, one of the leading investigators in this field, is using the fruit fly, Drosophila, for quantitative studies. Consistent with the previous experience of many workers, he observes that there are important quantitative differences in the response of different organisms to the same dose of mutagen. In his present studies, he is attempting to explain why such wide differences occur. He has measured the degree of reaction of a known mutagenic agent, ethylmethane sulfonate, with DNA and found this to be poorly reproducible and hence of little value as a mutagenic index. More recently, he has developed a somewhat different approach in which the mutagenic agent is injected into hamsters, and blood

plasma from the animals is fed to Drosophila. Sex-linked lethals induced in the sperm of the fly are then measured. He found a very high correlation between the amount of the chemical in the blood plasma and the frequency of lethals induced in the sperm. The results of this technique suggest that it may be used as a measure of mutagenic agents in the blood of animals or even man. Dr. Lee recognizes, however, that the amount of mutagenic agent used is very large relative to amounts that may occur in the environment, and he intends to carry out further studies to determine the lower limits of sensitivity of the test. It might be recognized also that this test, like the one described above, is a measure of lethal mutations and not of minor ones which may accumulate in the human genetic pool and become important over several generations. Thus, while progress is being made in test systems for mutagenesis relevant to man, no completely satisfactory system is available as yet.

Repair of DNA. An important aspect of mutagenesis which has a bearing on the persistence of genetic damage in the exposed organism and in subsequent generations is the repair of genetic lesions. Most cells contain enzymes which can recognize damage to DNA, excise the abnormal portions of the molecule, and insert a new fragment into the chain with all of the component bases in proper order for reproductive fidelity of the organism. Drs. Jerry Williams and John Little at the Harvard School of Public Health are studying this phenomenon to define the biologic mechanisms that maintain the fidelity of genetic material damaged by environmental agents or radiation. The process of repair is best documented and understood in bacterial cells subjected to UV radiation. However, the Harvard workers recognize that mammalian cell response is not analogous to bacterial responses. Therefore, their studies involve use of cultured human cell lines in order to assess the repair process in cells presumably relevant to man. They have identified two types of responses of these cells to UV radiation, distinguishable by differences in survival to a given dose of radiation and differences in responses to a chemical, proflavine, which tends to protect against UV. Their findings suggest that different cells respond to UV in different ways due to the presence of cellular components which constitute a protective screen and not to any inherent differences in susceptibility of DNA. They also find that age of the cell is a determining factor in the response.

Correlation of Carcinogenicity and Mutagenicity. Dr. Walter Troll and his colleagues at New York University have been engaged for several years in cancer research and, based upon several lines of evidence showing parallelism between the carcinogenic and mutagenic activity of certain compounds, advance the hypothesis that cancer initiation reflects a somatic mutation. Good correlation seems to exist between the capacity of certain toxic agents to produce mutations and cancer and their ability to react with DNA. The reaction with DNA can be assessed by its ability to act as a template in RNA synthesis (an enzymatic test). This test is much more sensitive than physical measurements of small changes in DNA. Should the correlation hold with all mutagens, this might well constitute a new test for mutagenic agents under some conditions and be a valuable contribution to some aspects of mutagenesis research. However, the test would not appear to be suitable for screening of human populations for mutations.

TERATOGENESIS AND OTHER REPRODUCTIVE EFFECTS

Birth defects associated with intake of thalidomide, polychlorodibenzodioxin, and mercury have brought the effects of environmental contaminants on reproduction forcefully to the attention of both the public and the scientific community. Thus NIEHS continues efforts to identify environmental agents that pose a risk in the reproductive process and to develop knowledge of fundamental aspects of the problem that will be useful in treatment and prevention of birth defects.

Heavy Metals. Various metals, including cadmium, indium, arsenic, cobalt, zinc, and mercury, have been shown to produce defects in embryos. Deficiency of zinc and manganese during pregnancy can also cause deformed offspring. Scientists with support of a NIEHS Center Grant at the University of Cincinnati have a very active program in this area, particularly with respect to essential metal deficiencies. Dr. Herbert Schumacher has evidence that congenital defects caused by thalidomide and salicylates are due to metal deficiencies induced by these compounds. He found that pregnant rats kept on a diet which was marginal with respect to zinc and fed thalidomide on days 8, 9 and 10 of pregnancy had pups with a 20 percent incidence of skeletal and soft tissue malformations and a fetal resorption rate of 25 percent. Severe retardation of intrauterine growth was also observed. Addition of zinc prevented the effect.

Congenital defects associated with methylmercury intoxication, as well as other aspects of the hazard of mercurials, are being studied by Dr. Modesto Yang at Michigan State University. He is concerned specifically with the stage during pregnancy when the fetus is at greatest risk. Preliminary experiments indicate that low levels of methylmercury fed on day 5 or 15 of gestation had no effect on the fetus. However, given on day 10, mercury produced a number of anomalies, including hemorrhage in various parts of the fetus and brains without definite structure. Dr. Yang's findings indicate, therefore, that there are rather specific periods during fetal development when the fetus is most susceptible to mercury.

Studies in other laboratories suggest that fetal susceptiblility to foreign agents at specific stages of development may be a general phenomenon. Dr. Raymond Harbison at Vanderbilt University, working with a known teratogen diphenylhydantoin has shown that the fetus is much more susceptible to the compound on day 9 than on day 15 or 17 of gestation. This is in accord with the time of maximum susceptibility found by Dr. Yang for methylmercury. Moreover, Dr. Harbison has shown that the greatest quantities of the compound pass the placental barrier and are absorbed by the fetus during gestational days 8 through 11.

Pesticides. Since the finding of birth defects associated with use of the herbicide 2,4,5-T, or, more specifically, the contaminant dioxin, a great deal of interest has centered around possible teratogenic effects of pesticides. Dr. James Gibson at Michigan State University has tested a number of herbicides for teratogenic effects, including DNBP (2-secbutyl-4,6-nitrophenol), paraquat, Bidrin and others. One compound, DNBP, was found to

be teratogenic when administered on gestational day 10. The anomalies were imperforate anus and lack of tails. There was also increased resorption of fetuses and reduced fetal size. Dr. Gibson is now attempting to determine the basic cause of the observed anomalies.

Dr. Harbison, whose work was referred to above, has devoted considerable effort to assessment of insecticides for fetal effects. Among others, he has studied parathion, heptachlor, chlordane and DDT. He found no effect of the chlorinated hydrocarbons on fetal development but showed that parathion is highly toxic to the fetus and induces a high incidence of resorption. Contrary to the findings for the teratogens mentioned above, the parathion effect was most severe during the latter part of gestation, the period of maximum organogenesis and maturation.

Phytotoxins. Certain toxins occurring as contaminants of food supplies also affect fetal growth and development. Vanderbilt University scientists Benjamin Wilson and Raymond Harbison have studied the toxins of sweet potatoes infected with Fusarium spp. and of grains infected with Penicillium rubrum. One of the toxins from sweet potato (ipomeanine) was found to be lethal to the fetus, with maximum effects toward the end of the gestational period. The Penicillium toxin, rubritoxin, was teratogenic, producing maximum effect when fed on days 8 through 10 of gestation.

BEHAVIORAL ABERRATIONS AND CNS EFFECTS

The clearly established interrelationships between central nervous system damage and behavioral aberrations and intake of lead, mercury and certain pesticides has focused attention of the public and behavioral scientists on these aspects of environmental health. NIEHS supports a number of studies probing both the basic action of toxic agents on the nervous system and the behavioral consequences of nervous system damage.

Lead. Studies on nervous system effects of lead by Dr. Richard Manalis at the University of Cincinnati show that lead ions produce neurological disturbances in both the central and peripheral nervous systems. In the periphery, lead accumulates at the endplate region of the muscle and, by elegant electrophysiological techniques, Dr. Manalis has shown that lead blocks electrical impulses at the junction, preventing proper transmission of messages from the brain to the muscle.

Studies by Dr. Albert Krall at the Medical University of South Carolina may help explain the marked difference in toxicity of lead in the adult and young. He has fed lead to mothers of suckling rats and looked for changes in brain metabolism and morphology in the young. His most important finding was that 25 days after birth the pups developed tremors, indicative of nervous disorders. Mitochondria and other indicators of metabolism showed no effect. However, he found that norepinephrine (NE), a hormone which effects muscle and heart action through the sympathetic nervous system, in the brain of pups from the treated mother was double that of normal pups. At 35 days the NE level returned to normal in those animals that survived. The reversal of toxicity and nervous symptoms corresponds to the formation of the blood-brain barrier in the rat at about 30 days after birth. Dr. Krall postulates that

the absence of the barrier or inadequate development accounts for the high susceptibility of the very young rat to lead and to the hormone EN.

Subtle deviations in behavior which will help in diagnosis of early and marginal effects of toxic agents are being studied in a number of laboratories with partial support from NIEHS. Drs. Joan Spyker and Sheldon Sparber at the Minnesota Medical School and Dr. Alan Golberg at Johns Hopkins University recently found that offspring of mice exposed to methylmercury on day 7 or 9 of pregnancy showed no signs of overt toxicity or of brain damage. However, they were able to demonstrate subtle behavioral effects. Pups from treated mothers took longer to begin exploration in an "open field" test. They did not move about as readily as their normal counterparts and showed a highly unusual backing phenomenon. They also showed one or more signs of neuromuscular impairment while swimming. They would often "freeze" in the water and float motionless with all four legs extended and askew.

Several pesticides also have been shown to produce abnormal patterns of behavior and reactions to stimuli. The pesticides include dieldrin, studied by Dr. George Burt at Oregon State University, and parathion, studied by Dr. Albert Casey at Texas A & M University.

EFFECTS ON THE IMMUNE SYSTEM

Numerous toxic agents and drugs suppress the immunological responses of the body to infectious agents and foreign tissues. This can be expected inevitably to lead to greater susceptibility to infectious agents and intensification of infectious and allergic diseases.

Certain toxic metals, notably lead, are immunosuppressants. Dr. William Buck at Iowa State University is investigating this effect and has found that mice given subclinical doses of lead in water for 30 days showed significantly greater mortality than those receiving no lead when subsequently infected with Salmonella. This finding is particularly relevant to chronic lead poisoning in children and infants, whose susceptibility to lead intoxication is much greater than that of adults and whose immunological responses and defenses are less developed.

Beryllium is of considerable interest because it has a quite different effect of the immune system. Dr. Andrew Reeves, an NIEHS grantee at Wayne State University, has shown that some inhaled beryllium becomes bound to proteins. The protein-Be complex is immunogenic and triggers the formation of antibodies. As a result, new tissue forms around the site of beryllium deposition giving rise to the fibrotic and sclerotic lesions characteristic of berylliosis.

In addition to the effects of man-made chemicals on the immune system, NIEHS also supports studies on toxins of natural origin. An example is the work of Dr. James Barrett at the University of Missouri on the immunochemistry of the recluse spider toxin. The recluse spider has become increasingly prevalent in the midwest in recent years and appears to be moving east. Dr. Barrett is attempting to characterize the venom and to study its immunologic properties. In work with guinea pigs, he has shown that the venom is

immunogenic and, based on work completed to date, he feels that immunoassay procedures can be developed to diagnose and verify bites. He has also had some success in developing antisera useful in modifying the course of ulceration that follows the bite. At the present time, he is attempting to purify and identify the components of the venom and feels that more effective antisera can be developed when purified toxin is available.

EFFECTS ON SPECIFIC ENZYMES AND OTHER CELL COMPONENTS

NIEHS supports a substantial number of projects which seek to identify the changes at the molecular level brought about in cells by toxic agents. Some of these have been mentioned previously in connection with broader manifestations of effects in the form of mental and behavioral anomalies, carcinogenesis, pulmonary diseases, etc. Thus, a few examples will suffice to illustrate the nature of work in this area and the goals of the Institute.

Heavy Metals. Several studies at Oregon State University on the effects of toxic metals and pesticides are being supported through a Center grant. Dr. Sheldon Wagner reports that recent medical observations implicate lactose intolerance as a symptom of acute mercurial intoxication. Lactose is milk sugar digested by the enzyme lactase. Inactivation of this enzyme prevents lactose digestion, resulting in abdominal cramps and diarrhea. Dr. Wagner's studies show that the enzyme is indeed inhibited by mercurials and probably accounts for the observed symptoms following ingestion of milk.

Several studies in progress show that metal pollutants bind with a wide spectrum of proteins in various parts of the body. Experiments by Dr. Paul Simpson at Oregon State University using X-ray analysis show that mercurials are bound to proteins at a number of ionic sites. Dr. Walter Farkas at the University of Tennessee has demonstrated similar binding of the lead ion. These studies are illustrative of many which indicate indiscriminate interaction of toxic metals with proteins. Clearly, this complex interaction pattern increases manyfold the problem of finding a primary site of action of metals. Nevertheless, progress has been made in identifying rather specific effects that, if not causal in disease symptoms, are undoubtedly contributory to degenerative processes over long periods of exposure. For example, it has been found that inhibition of protein synthesis is among the first recognizable effects induced by lead and mercury. This is consistent with damage to the nervous system where replacement of protein is normally a very slow process compared to that in other organs, and provides insight into the cumulative damage resulting in the subtle behavioral changes induced by both of these metals as noted elsewhere in this report.

Lead and mercury compounds have also been known for a long time to cause kidney damage. Through basic studies on the interactions of these compounds with proteins, the reasons for such damage are becoming increasingly clear. Work by Dr. E. C. Foulkes at the University of Cincinnati indicates that lead and mercury tend to accumulate at the lining of the kidney tubules where they react with the proteins of the membrane. This interfers with the reabsorption of amino acids and sugars which normally pass from the blood stream into the lumen of the tubules and are then selectively reabsorbed as they pass through the tubule. Failure of the reabsorption mechanism is called the Fanconi syndrome.

The reabsorption of amino acids is a process which requires energy. The energy is produced by cells that line the kidney tubules. Thus, it is clear that lead and mercury affect reabsorption not only by passive binding to the tubular lining but also by interfering with the generation of energy necessary for transport. Experiments by Dr. Goetz Richter, supported by an NIEHS grant, and independently by others, help provide an explanation of this phenomenon and to provide further clarification of the disease process. Dr. Richter finds that after a single injection of lead in rats some of the lead becomes bound to mitochondria in the liver and kidney. There is a great deal of evidence from many sources that lead and mercury in very minute quantities in the mitochondria inhibit the enzymes necessary for energy generation. Thus, the molecular basis of kidney damage is fairly clear.

Pesticides. Similar studies with the same objectives are being supported in the pesticide field through the Center Grant at Oregon State University, a project grant at NC State University, and a number of grants to individual scientists. At Oregon State, Dr. R. Hague has demonstrated that a number of insecticides bind to phospholipids. A number of the more important insecticides are nerve poisons and concentrate in nerve cells. Dr. Hague's work suggests that they concentrate in these cells because of their affinity for the phospholipids. Exactly why interaction with phospholipids causes the paralysis characteristic of the actions of these insecticides is not yet understood but groundwork laid by basic studies on molecular interactions provides a solid foundation for further work toward full understanding of the action and consequences of these and other neurotoxins. At North Carolina State University, studies by Dr. Frank Guthrie and his colleagues indicated that dinitroaniline herbicides have a pronounced effect on growth and metabolism in both plants and animals. They have found that these effects can probably be explained by the fact that the compounds in minute quantities interfere with generation of chemical energy in mitochondria much the same as lead and mercury act in the mitochondria of kidney cells. They believe, however, that the interactions at the molecular level are different from those of the metals but produce the same overall effect. Energy generation involves a number of different enzymes and steps so that interruption of the process at different points and by different mechanisms may occur with the same result. They have found also that the herbicides interfere with production of energy in plant chloroplasts during the process of photosynthesis and postulate that this may be the basis for their lethal effects on plants.

BODY DEFENSE MECHANISMS

Studies on body defense systems constitute one of the major activities of biochemists and toxicologists during the past decade. Despite remarkable progress, much remains to be done and NIEHS supports research toward further clarification of all of the mechanisms mentioned and evaluation of each mechanism with respect to the more important specific hazardous agents in the environment.

Lung Clearance Studies

Particulates, as well as gaseous chemicals, are clearly a major factor in lung diseases. The probability of emphysema, bronchitis, pneumoconiosis, cancer,

silicosis, and even infectious diseases resulting from inhalation of particulates depends to a great extent on the efficiency of pulmonary clearance. The alveolar macrophage, a mobile, ameboid cell found in the surface of the air passages, has a central role in the clearance process. Therefore, it is necessary to understand macrophages in order to understand pulmonary defenses. Using newly developed techniques, Dr. Brain of Harvard University has shown that alveolar macrophages do not originate in the lungs but are entirely of hematopoietic origin; that is, they originate in the bone marrow. Thus, agents that affect the bone marrow may also affect the clearance of particles in the lung. In accordance with this, Dr. Brain has shown that the number of macrophages is decreased by radiation and required about 28 days to return to normal. Other studies on the effect of particle inhalation on alveolar macrophage phagocytosis rates show that macrophages of hamsters exposed to iron oxide particles take more time to ingest subsequently inhaled particles. This shows that the process of phagocytosis slows down as the particle load increases. These kinds of studies are expected to provide useful information about the capability of clearance systems and to provide an index of risk vs. air particle density. They also further our understanding of associations between multiple stresses on interrelated and inter-dependent systems in the body.

Dr. Joseph Watson at the University of Pittsburgh is also studying the role of macrophages in respiratory tract clearance. He has devised an ingenious technique for determining the number of phagocytic cells available for clearance. Most workers in this field have used the total number of cells from lung washings as the sole criterion for characterizing phagocytic action and capability. Dr. Watson uses an aerosol of minute iron particles which is inhaled. He then lavages the lung in the usual way; but subsequently he uses a magnet to collect cells which actively ingest the iron particles. This method, he believes, gives a more valid measure of the phagocytic capability of the lungs and will enable him to determine more accurately changes in this capacity in relation to exposure to various air pollutants, age and concomitant respiratory diseases.

Insecticide Metabolism. Metabolism of insecticides and other foreign compounds is an extremely active area of research. At least 20 NIEHS grantees are engaged in one or more aspects of this problem. Thus, the following is a summary of accomplishments shared by many individual scientists.

It has been shown that the more important enzymes in insecticide metabolism are located in submicroscopic bodies of cells called microsomes found primarily, but not exclusively, in the liver and kidney. The enzyme system is made up of a number of components which act in concert to insert oxygen into the molecule, at various ring positions, at nitrogen atoms and at other site or side chains. Enzymes of this system are commonly called mixed function oxidases. These systems are present in almost all forms of life, including plants and insects as well as mammals. However, they vary considerably from organism to organism and with respect to age of the organism. It has been shown that certain compounds cause an increase in activity of the system. Among these are phenobarbital and a number of pesticides.

Conversely, toxicants have been found which inhibit the MFO system. In general, such compounds cause accumulation of toxicants and raise their

toxicity above that normally expected. Several such compounds, e.g., piperonyl butoxide, have been known for many years to increase the toxicity of DDT and other insecticides. New compounds which act in this manner have been found recently and are being studied by NIEHS grantees.

In certain cases, the MFO system serves to enhance the toxicity of toxic agents. A notable example is parathion. Through the action of the MFO system a sulfur atom is replaced by an oxygen atom to form the metabolite paraoxon, which is markedly more toxic than parathion. Related insecticides such as malathion and EPN are also oxygenated by the system, with a concomitant increase in mammalian toxicity. Certain other important insecticides, e.g., dimethoate, dicrotophos, phosphamidon, contain a side chain connected to a ring through a nitrogen atom. Largely through the studies of Dr. Robert Menzer, an NIEHS grantee at the University of Maryland, it has been demonstrated that these compounds are degraded through a series of steps in involving removal of the side chain and oxidation of the nitrogen atom and eventually elimination of the degraded products through the kidnesy. The MFO system can also be induced to metabolize larger than normal amounts of these types of compounds. This has the effect of increasing the tolerance of the exposed organism.

Many natural as well as man-made products are metabolized by way of the MFO systems. Recent studies by Donald Lee at Oregon State University show that aflatoxins produced by the mold Aspergillus flavus are rapidly oxidized in mammalian liver to give derivatives which are not carcinogenic.

Very recent studies have shown that the mammalian body also has a mechanism of defense against toxic heavy metals. Exposure to such ions as Cd, Cu or Hg at less that acutely toxic doses triggers synthesis of a protein called metallothionein which strongly binds metal ions and prevents their interaction with functional proteins. The nature and mode of induction of this protein is not yet understood and NIEHS has recently awarded a grant to Dr. Sara Bryan at Louisiana State University for studies to develop more information about these aspects of the substance.

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